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DIRECTIONAL TUNING OF CORTICAL NEURONS FOR THE CASE OF BIMANUAL MOVEMENTS

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Biomedical Engineering

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Abstract

Although the majority of our upper-limb movements are performed with two arms, only a few neurophysiological studies have addressed neuronal mechanisms of bimanual motor control. In particular, little is known about directional tuning of cortical neurons during bimanual motor tasks. This contrasts with the vast literature on the same topic for unimanual movements. Georgopoulos and his colleagues pioneered the description of neuronal mechanisms of unimanual movements in terms of directional tuning curves, where firing rate of a neuron is expressed as a function of reach angle for center-out movements. According to Georgopoulos, individual neurons in the motor cortex are broadly tuned to movement direction, with the firing rate reaching a maximum value for the direction called preferred. This tuning pattern is well approximated by a cosine curve. No such description has been developed for bimanual movements. The main objective of the project is to address this gap in knowledge. For this purpose, an analysis will be presented of neuronal recordings performed in rhesus monkeys trained to perform bimanual tasks. Multiple linear regression fitting methods will be used to describe the dependency of neuronal rates on the movement directions of two arms, comparing the results obtained with each method. Neuronal tuning for unimanual movements (performed with either the left or right arm) will also be compared with the tuning for bimanual movements, testing whether bimanual tuning could be derived from the tuning for each arm tested separately, or if it is principally different from the dependencies for unimanual movements. The results from this work will eventually lead to improved neural decoding algorithms that could be utilized in brain-machine interfaces.

Resumen

A pesar de que la mayoría de los movimientos que realizamos con los miembros superiores sea con los dos brazos, pocos estudios neurofisiológicos han analizado los mecanismos neuronales del control motor bimanual. En particular, sabemos poco acerca del ajuste direccional de las neuronas motoras durante movimientos bimanuales. Esto contrasta con la extensa investigación que se ha realizado sobre el mismo tema para movimientos unimanuales. Georgopoulos y sus colaboradores lideraron la descripción de movimientos unimanuales en términos de curvas de ajuste direccional, donde la actividad neuronal se expresa en función del ángulo del movimiento del brazo. Según Georgopoulos las neuronas del cortex motor se ajustan de manera "amplia" a la dirección del movimiento, siendo máximo el ritmo de activación de la neurona para movimientos realizados en la "dirección preferida" de la neurona. Esta curva de ajuste se aproxima a un coseno. Una descripción de este tipo para movimientos bimanuales no ha sido realizada aún. El objetivo principal del proyecto es abordar esta laguna de conocimiento. Para ello se analizarán grabaciones neuronales de macacos Rhesus durante movimientos bimanuales. Se utilizarán varios modelos de regresión linear múltiple para analizar la relación entre la actividad neuronal y la dirección del movimiento de los brazos. Asimismo se contrastarán los resultados obtenidos con los diferentes métodos. El ajuste direccional de las neuronas durante movimientos unimanuales será comparado con el obtenido para movimientos bimanuales. Específicamente, se estudiará si el ajuste direccional en movimientos bimanuales puede derivarse del ajuste direccional de cada brazo durante movimientos aislados, o si es mayoritariamente independiente de la representación neuronal durante movimientos unimanuales. Los resultados de este trabajo podrán contribuir al desarrollo de mejores algoritmos para descodificar la actividad neuronal, los cuales son una parte esencial de las Interfaces Cerebro-Computadora.

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Acronyms

MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
SPECT	Single Photon Emission Computed Tomography
EEG	Electro-encephalography
ECoG	Electro-corticography
M1	Primary Motor Cortex
S1	Primary Somatosensory Cortex
PPC	Posterior Parietal Cortex
SMA	Supplementary Motor Area
CNS	Central Nervous System
PNS	Peripheral Nervous System
BMI	Brain Machine Interface
BFPD	Best-Fit Preferred Direction
PD	Preferred Direction
UKF	Unscented Kalman Filter
BC	Brain Control
UR	Unimanual Right
UL	Unimanual Left
BR	Bimanual Right
BL	Bimanual Left
PETH	Peri-Event Time Histogram

Glossary of Terms

Bimanual Movement: Movement performed simultaneously with the two arms

Complex Movement: Bimanual movement where the direction followed by each of the two arms differs by 90 degrees

Contralateral: Body side opposite to the hemisphere where a given neuron is located

Directional Tuning: Corresponds to the specificity in the response of a neuron to the direction of movement

Ipsilateral: Body side of the hemisphere where a given neuron is located

Opposite Movement: Bimanual movement where the two arms move in opposite directions

Preferred Direction: Movement direction for which the response of a given cell is highest

Parallel Movement: Bimanual movement where the two arms move in parallel

Tuning Depth/Tuning Amplitude: Amplitude obtained when performing a multiple linear regression of the signal-to-noise ratio of neuronal responses

Tuning Degree of a Neural Population: Percentage of neurons within the population that are directionally tuned

Unimanual Movement: Movement performed with a single arm

1 Introduction

1.1 Motivation

We have seen in the last few decades an explosive development of techniques that allow us to record, visualize and analyze brain activity. These include imaging techniques such as MRI, PET or SPECT and techniques based on the recording of the magnetic and electrical fields associated with brain activity such as EEG, MEG, ECoG or intracortical electrode arrays. These techniques have vastly increased our understanding of information processing in the brain. In particular we are now able to observe which brain regions are activated and how they are activated when a particular action is executed or a particular mental imagery is performed.

When considering neural correlates of voluntary movements, EEG, ECoG and implanted electrode arrays are the most commonly employed techniques. They differ in invasiveness as well as sensitivity. EEG allows to easily and non-invasively record brain potentials, however the signal is very noisy and the recorded signal is a combination of many individual action potentials, which makes it impossible to use the signals for finely tuned movements. On the other hand, implanted electrode arrays, the most invasive procedure, allow now to record the activity of individual neurons for samples of up to two thousand cells [1]. This technique opens now the door for real-time monitoring of the firing patterns of spatially extended neural networks, combining both precision to detect individual neuron spikes and large-scale spatial distribution to study the behavior of the network. For these reasons it is well suited for Brain Machine Interfaces (BMIs) that allow the brain to directly communicate with external machines. In the particular case of movement, these systems allow the user to send movement commands encoded in the brain to an external device: prosthetic arm, exoskeleton

and others. These systems therefore offer important possibilities for people with neurological disorders impeding movement. In particular mind-controlled prosthetic arms allow people with tetraplegia to perform some simple movements such as reaching for a drink, helping them in this way to regain some independence. Systems allowing locomotion in paraplegic people pose some important challenges related to balance, control and freedom range of movements and will therefore take longer to become widely used. This field is evolving at a fast rate, and new experiments and improvements continuously emerge.

BMIs for movement commonly rely on the recording of motor cortical cells but sometimes also include cells from the somatosensory and posterior parietal cortices. In particular recent papers seem to indicate that the use of neurons from the posterior parietal cortex (PPC) may yield smoother movements that are also easier to generate for the person controlling the BMI [2]. Different algorithms exist to decode movement intentions from the neural activity and so far most of the BMI systems that have been developed for upper limbs only allow decoding movements of a single arm at a time. Since most of the movements that we routinely perform include the simultaneous movement of the two arms, there is an important gap in both our understanding of motor neurophysiology and BMIs that prevent the development of clinical devices that assist disabled people to perform a wide range of useful arm movements. For both practical and theoretical reasons it is therefore important to determine how bimanual arm movements are encoded in the brain.

Much research has focused on the mathematical description of how neurons respond to unimanual movements. Georgopoulos first proposed in 1982 that neurons responsive to movement tend to have a preferred direction of movement for which their discharge rate is highest [3]. He found that neuronal response across all movement directions can be approximated by a cosine function. Since then, numerous papers in the field utilized this idea [4] [5] [6]. However some other papers have used different approaches and have employed multiple regression techniques [7]. In most cases the method involves across-trial averaging of the response and it is still not understood whether this averaging of the data captures the major characteristics of neuronal tuning or trial-to-trial variability should be taken into consideration as well.

Despite the efforts directed to deciphering unimanual movement encoding, it is still not well understood how bimanual movements are represented in the brain. Some groups have compared the activity patterns of unimanual and bimanual movements [8], although no clear mathematical description has yet been derived. Given the low number of papers devoted to

this subject, more experiments are needed to reproduce and validate the results as well as to produce new knowledge.

1.2 Objectives

The current project will attempt to complement our understanding on the encoding of bimanual movements in cortical neurons. The project will also look to summarize and compare different approaches that can be used to extract behavioral parameters from neural recordings.

The specific objectives of this Thesis can be summarized as follows:

- Study of Different Models of Neuronal Tuning to Movement Direction:
  - Standard cosine-fit approach with across-trial averaging
  - Multiple linear regression without trial averaging
  - Comparison of the results obtained with the different models
- Study of Bimanual Movement Encoding by Cortical Neurons:
  - Exploration of the difference in tuning properties for a given neuron for unimanual versus bimanual movements
  - Linear fitting of the bimanual response to movement direction
  - Neuronal representation of the complexity of bimanual movements

1.3 Context of the Project

This Bachelor Thesis was conducted in collaboration with the Duke Center for Neuroengineering (Durham, USA), headed by Dr. Miguel Nicolelis. It continues the internship that I completed in the summer of 2014 at Duke. During my internship, I became familiar with BMIs and neural data processing and analysis. My internship project addressed the dynamics of neurons during monkey reaching tasks from a network perspective and doesn't overlap with the content of this Thesis.

Duke researchers developed in 2013 the first BMI that enabled for bimanual movements [9]. In this study, neural activity was decoded using a filter that represented movement parameters

(arm positon and velocity) as a function of neuronal activity over a 1s window that preceded the measurement of movement parameters. This study also demonstrated that BMIs could be used for bimanual tasks in subjects who are unable to produce arm movements. The collected neural data was not fully analyzed. This data offers a wealth of information regarding the relationship between neural firing patterns and the type and direction of performed movements. For this reason, Mikhail Lebedev, Senior Researcher at the Duke Center for Neuroengineering, asked me to continue my data analysis with the exploration of this dataset in order to get further insight on the encoding of bimanual movements and in this way enrich our knowledge in the field.

The totality of the data used in the project was thus acquired in 2013 during the experiment of the Duke Center for Neuroengineering attempting to develop the first bimanual BMI. The main guidelines of the project have followed the interests of the Center and my work has continuously been supervised by Mikhail Lebedev, who guided me through all the steps in this analysis.

2 State of the Art

In order to understand neuronal directional tuning and its role in the control of voluntary movements we need to understand first how neural motor commands are generated. We will thus start with a review of the basic concepts of the structure of the nervous system and neural motor systems.

2.1 Nervous System and the Brain

Our bodies are extremely complex structures with different levels of organization such as organs, tissues and cells. A recent paper published in 2013 proposed an estimation of the number of cells in a human body to be on the order of 10^{13} [10]. The coordination of the actions of such a large number of components is managed by the nervous system.

The nervous system is defined as the tissue containing nerve cells or neurons. These cells, thanks to their specialized structure, can exchange messages and communicate with other cells. Neurons include the following parts: a cell body where the nucleus is located, several branched extensions called dendrites and one long slender extension called the axon (Fig 1). The dendrites receive, process and integrate signals coming from other neurons whereas the axon sends the output signal to other neurons. The signal is electrical and its transmission is enacted by the generation of action potentials, short-lasting variations of the cell membrane voltage potential. The transmission of signals between neurons is electrochemical (synaptic transmission) or electrical (gap junctions).

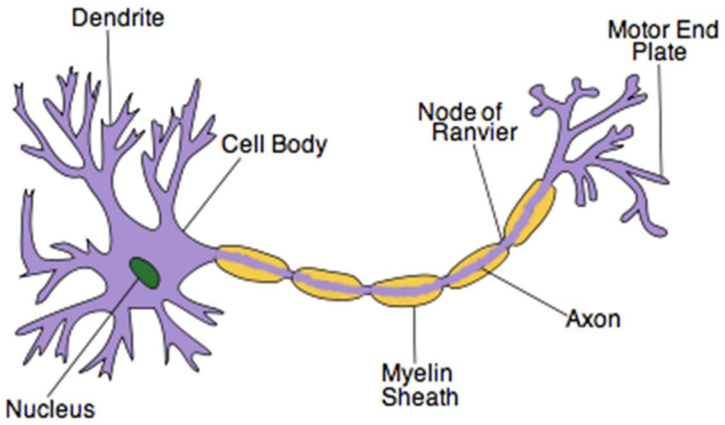


Fig 1: Schematic of the structure of a neuron (http://ibguides.com/)

Consequently, thanks to the extraordinary characteristics of its cells, the nervous system can transmit signals across the body and ultimately coordinate both voluntary and involuntary actions.

The nervous system is subdivided into the Central Nervous System (CNS) and the Peripheral Nervous System (PNS). The CNS comprises the brain, including the cerebrum, the cerebellum and the brainstem, and the spinal cord. The PNS consists of sensory neurons located in nerves and ganglia located outside the CNS. In simple terms, the CNS is the main information processor and the PNS works as a communication pathway between limbs and organs and the CNS.

Within the CNS, the brain (i.e. the part located inside the skull) performs higher-order functions whereas the spinal cord transmits signals between the brain and the PNS, and performs low-order functions often called reflexes. The brain itself can be subdivided in several regions having different, although not immutable, functions (Fig 2). Two of those regions, called the frontal lobe and the parietal love, are considered in our study. The frontal lobe, located in the frontal region of the brain, processes conscious thoughts. An important area of this lobe is the motor cortex which controls voluntary movements. The parietal lobe, positioned behind the frontal lobe, handles sensory information. An area in this region, called the somatosensory cortex, processes tactile and proprioceptive information.

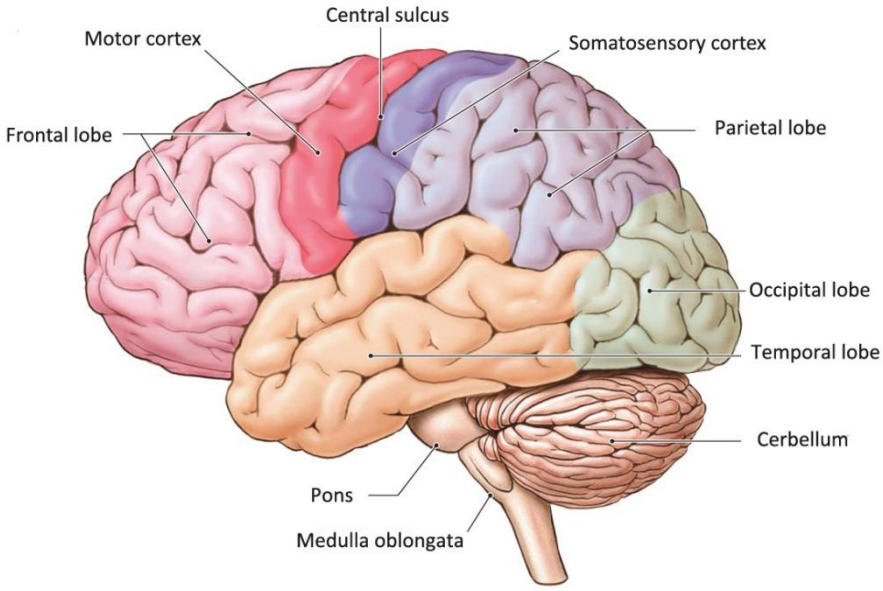


Fig 2: Lobes and areas of the brain [27]

In more detail, the following areas in the frontal and parietal lobes are considered in the present study.

- Motor Areas:
  - *Premotor Cortex*: its functions are complex and not fully understood although it seems to perform functions such as motor planning and sequencing
  - *Primary Motor Cortex (M1):* main neural pattern generator for movement execution
  - *Supplementary Motor Area (SMA):* its functions are diverse but it seems to have a direct impact on the coordination of both sides of the body

- The Somatosensory Cortex:
  - *Primary Somatosensory Cortex (S1)*: the main area receiving sensory information from peripheral somatosensory receptors
  - *Posterior Parietal Cortex (PPC*): plays a major role in generating voluntary movements; it integrates information about the position of the parts of the body involved in the movement and its relative position to external objects

Both the motor and somatosensory cortices are known to have a map of the different locations of the body, which is sometimes referred to as the homunculus (Fig 3). This means that only specific regions of the cortex are strongly and directly related to the movement of a part of the body.

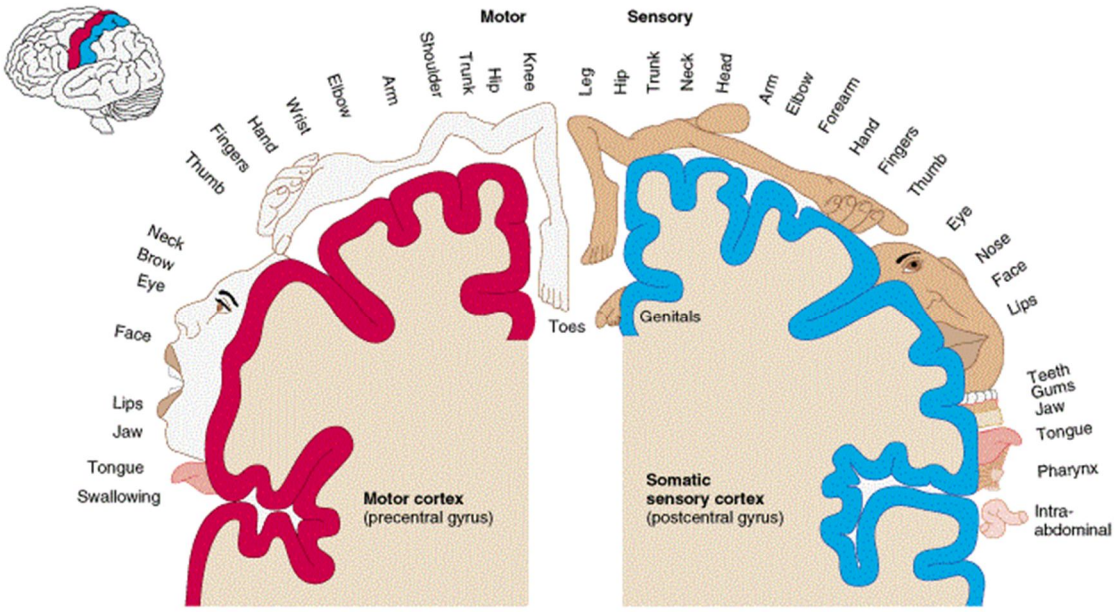


Fig 3: Homunculus in the motor and somatosensory cortices (http://www.ehinger.nu/)

For any kind of voluntary movement, a neural command is generated in the specific area of the motor cortex and eventually transmitted via the brainstem and the spinal cord to the appropriate skeletal muscles that will contract and perform the movement. Receptors in the skin and within the muscles will then allow a sensory feedback to be sent back to the somatosensory cortex carrying information concerning the performed movement (Fig 4).

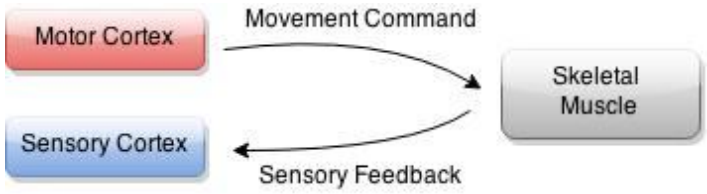


Fig 4: Simplified nervous pathway for voluntary movement

For manual movements specifically, a neuron activation pattern in the brain will command the contraction or relaxation of several muscles of the arm such as the triceps, biceps and the forearm flexor. To command such a movement several muscles have to be controlled and coordinated simultaneously. A large population of neurons is involved in generating this multiple degree-of-freedom action. But how is the intended movement actually encoded in the activity of the population of neurons?

2.2 Unimanual Movement Encoding in the Brain

Despite the research efforts that have been done in the last several decades, scientists have not yet reached a complete agreement on how neural control of movements is achieved. Is the activity of each muscle encoded so that some neurons directly dictate the contraction or relaxation of a specific skeletal muscle? Another hypothesis states that neurons do not encode muscle activity but rather represent the kinematics and kinetics of the intended movement, in a way that a group of neurons represents a somewhat higher-order motor parameter such as movement direction. Still some other researchers argue that none of those two hypotheses is entirely correct. They support the so-called Dynamical Systems Perspective where motor (and other) actions are believed to be produced by state transitions in neuronal populations, which can be described by dynamical system equations [11].

Although this debate is still ongoing, it is generally accepted that neurons represent (or at least are correlated to) parameters of the movement such as velocity and direction. The relationship between neuronal activity and motor parameters is called neuronal tuning.

2.2.1 Directional Tuning and Population Coding

The phenomenon of neuronal tuning has been known for the last decades. Neuronal tuning is defined as the ability of certain neurons to represent a behavioral parameter. This parameter could belong to the sensory, cognitive or motor domain. A popular example illustrating this are retinal cones. These cells are tuned to light spectrum. This means that they are more sensitive to a given range of the electromagnetic spectrum. In particular we can distinguish between three types of cones each having different pigments and thus responding to different

wavelengths. The activation pattern of the three populations of cells allows the brain to identify a given color [12]. For cells in the motor cortex, tuning is usually the sensitivity of some cells to a particular velocity or direction [13]. Several studies have shown strong correlations between the activity of the neurons and the direction of the movement [3]. This property is referred to as directional tuning. There is a vast literature on directional tuning.

Georgopoulos first introduced this idea in 1982 in his famous paper. Together with his colleagues, they studied the response of more than 300 single cells in the motor cortex of rhesus monkeys. Their monkeys performed arm movements in different directions. Georgopoulos and his colleagues reported a clear tendency of the neurons to vary their firing rate as a function of the direction of movement (Fig 5).

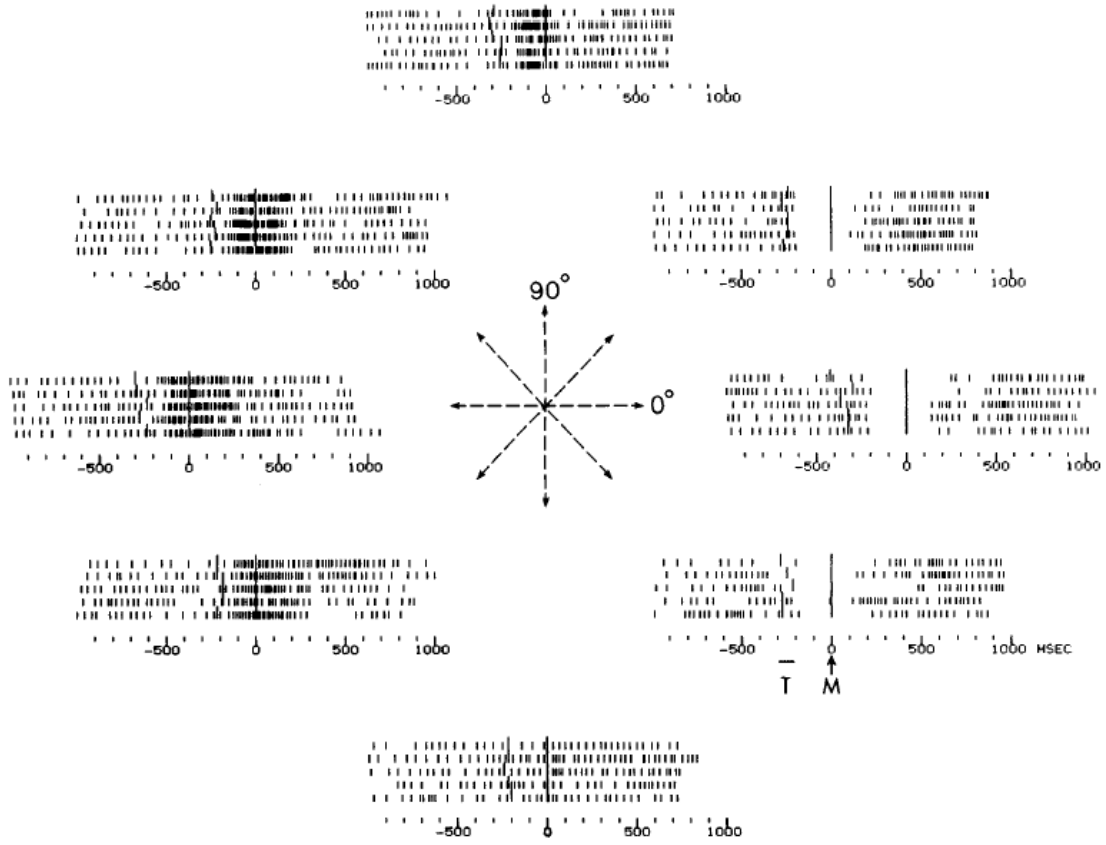


Fig 5: Directional Tuning by Georgopoulos. Spike trains of one neuron during unimanual movements in eight different directions **[3]**. For each direction, the response of five different trials is shown for a 2s time window around movement onset. This neuron is more active in movements with directions between 90 and 225 degrees.

According to their results, which have been verified in several other experiments, a neuron has a preferred direction and fires accordingly: the firing rate is maximal when the movement direction coincides with the preferred direction and minimal when the movement direction is

opposite to the preferred direction. As a result the neuron exhibits a bell-shaped directional tuning curve which can be fitted with a cosine curve (Fig 6).

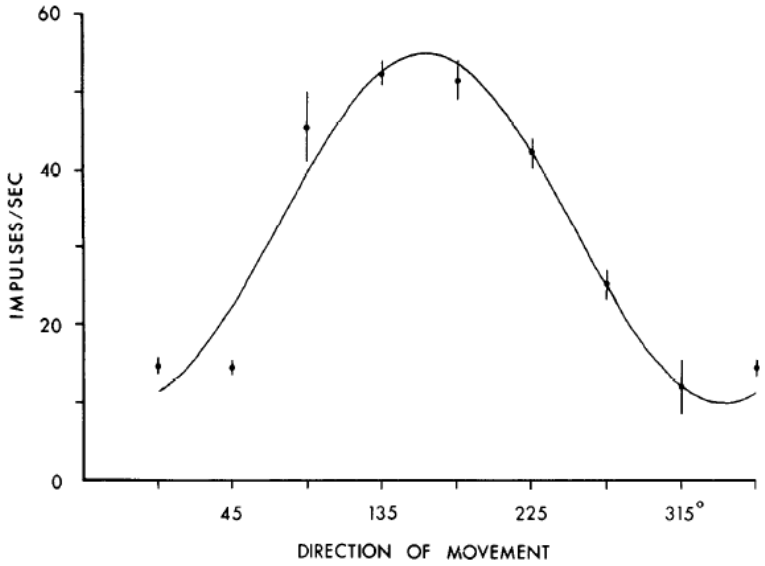


Fig 6: Cosine-Fit of neuronal responses.
Firing frequency of a neuron for movements performed in different directions **[3]**

The equation for the fitting is:

$$D = b_0 + b_1 \sin\theta + b_2 \cos\theta$$

Or equivalently

$$D = b_0 + c_1 \cos(\theta - \theta_0)$$

where b_0, b_1, b_2 and c_1 are regression coefficients and θ_0 is the preferred direction.

In this context neurons are considered to be broadly tuned to movement direction. This means that they do not respond very specifically to their preferred direction, but rather gradually decrease their activity as movement direction deviates from the preferred one. Additionally, neurons represent direction in a stochastic way: their rates are highly variable even if the movement direction stays constant.

Georgopoulos et al. also found that different neurons vary in their preferred direction so that the tuning curves of different cells are partially overlapped. According to their results the distribution of preferred directions is not homogeneous across space (Fig 7). In particular they

found a higher percentage of cells with preferred directions around 45° and a lower one for directions around 225°.

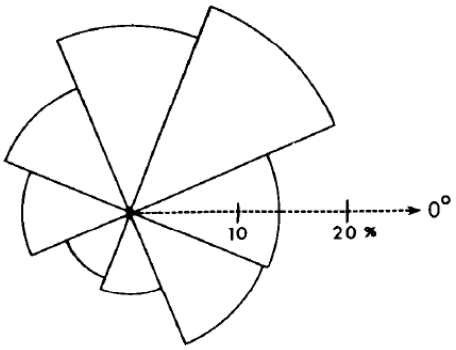


Fig 7: Distribution of preferred directions of the motor neural population across space [3].
Radial axes indicate the percentage of neurons whose preferred direction lies in the specific region.

In this way, a movement in the 45° direction is governed with major activation of cells whereas movement in the 225° direction is governed by major cell inhibition. A similar population with similar preferred direction was thus engaged in both movements: with an increase in the activation frequency and with a decrease in the activation frequency correspondingly. They suggest therefore that the movement in a desired direction is achieved thanks to the cooperation of a population of cells with overlapping tuning curves. This is what they called the Population Code.

2.2.2 Population Code

To study the hypothesis of the Population Code, Georgopoulos and his colleagues represented individual cell contributions as weighted vectors pointing in the preferred direction of each cell [14] [15]. Again the discharge rate was given by the following equation:

$$d(M) = b + k \cos\theta_{CM}$$

where θ_{CM} is the angle formed between the movement direction and the preferred direction. Then the *i*th cell has a contribution with a magnitude of

$$w_i(M) = d_i(M) - b_i$$

And accordingly a weighted vectorial contribution is given by:

$$N_i(M) = w_i(M)C_i$$

where C_i is a vector pointing in the preferred direction of the cell *i*. Finally the population vector for a given movement M can be calculated as the sum of all weighted contributions:

$$P(M) = \sum_{i=1}^{224} N_i(M)$$

The population vector is thus defined as the sum of all cell vectors, whose direction is given by the preferred direction of the cell and whose magnitude corresponds to the firing rate [6]. They found that the population vector can accurately predict the direction of the intended movement (Fig 8).

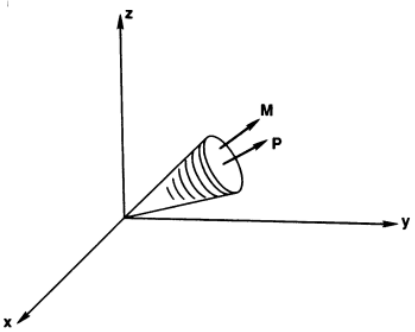


Fig 8: Population Code Vector. Predicted Population Vector P and real movement direction M. The cone describes a 95% confidence interval for P **[6]**

2.3 Neural Encoding of Bimanual Movements

Many studies have attempted to unravel how unimanual movements are encoded by the neurons. However several papers have reported more complex tuning of single cells for bimanual movements. Differences have been found in the representation of unimanual and bimanual movements by neurons (Fig 9) [6] [16]. Additionally it is not obvious that the concept of Population Vector works in the case where several directions are represented simultaneously, as is the case for bimanual movements.

In one study Steinberg et al. found that the Population Vector approach can accurately predict bimanual movements [17]. They showed that the preferred direction of neurons was relatively well preserved for both unimanual and bimanual movements (Fig 10).

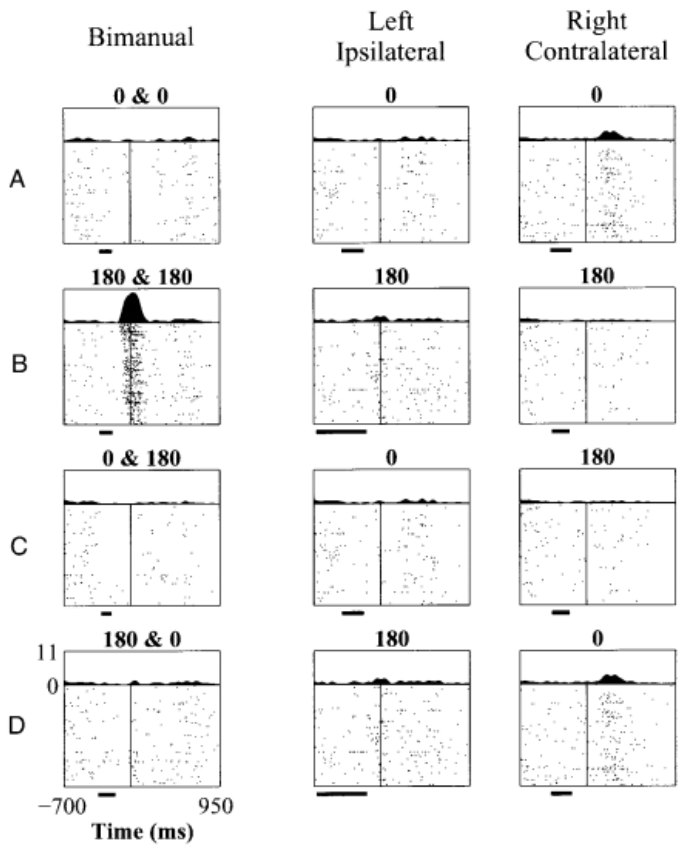


Fig 9: Bimanual vs Unimanual Responses. Spike trains and smoothed response of an M1 cell to bimanual and unimanual movements in different directions **[17]**

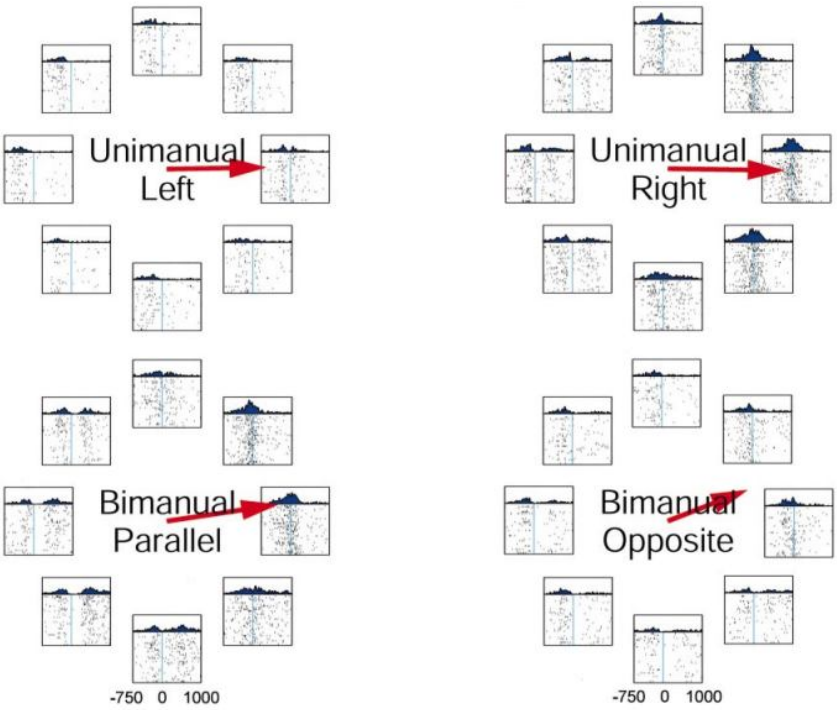


Fig 10: Preferred Direction of cells for different movement types. An example of a cell that maintains its preferred direction (red arrow) across different movement types **[17]**

For their study they performed both unimanual and bimanual opposite and parallel movements with four target positions. Bimanual parallel movements are defined to be those where the arms move in the same direction whereas in opposite movements the direction of movements differs by 180°. To study the preferred directions of cells they used the cosine approximation introduced in previously published papers [15]. To study variations in preferred directions for different types of movement they characterized trials for a given neuron as unimanual ipsilateral, unimanual contralateral, bimanual parallel or bimanual opposite.

Unimanual ipsilateral trials correspond to movements of the arm that is located in the same side of the body as the neuron (i.e. neuron in the right hemisphere and movement of the right arm). Unimanual contralateral trials correspond to movements of the arm that are on the opposite side of the body to that of the neuron (i.e. neuron in the right hemisphere and movement of the left arm) (Fig 11).

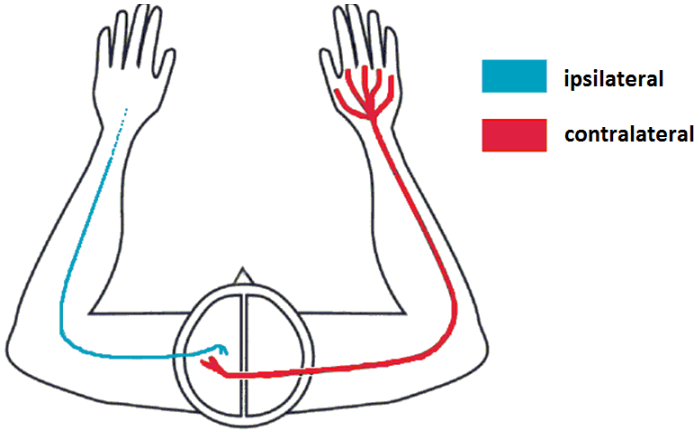


Fig 11: Definition of contralateral and ipsilateral movements [28]

In order to assign a single preferred direction to each cell they calculated the so-called best-fit preferred direction (BFPD). As they themselves explained, they could have calculated the preferred direction (PD) from each of the four movement types and then take the average. However this approach would not have taken into account the variations in response magnitude across the different movement conditions. Therefore they calculated the preferred direction by doing least-squares fit across all four movement conditions imposing the restriction that the preferred direction is constant for all of them. The equation used for the fit is as follows:

$$y_T(\theta) = a_T + c_T + \cos(\theta - \theta_0)$$

where a_T and c_T are specific for the movement type and Θ_0 is the preferred direction. In bimanual opposite trials the angle was defined to be that of the arm producing a stronger response in the cell. They found that most of the cells are at least broadly tuned to one particular type of movement. But more importantly they also found that the preferred direction of single cells is similar in different movement types (Fig 12). In particular, bimanual movements had the greatest similarity with contralateral unimanual movements. These results can be expected since it is widely known that the hemispheres in the brain command the contralateral side of the body and therefore the left hemisphere will determine to a greater extent the movement of the right arm than the right hemisphere.

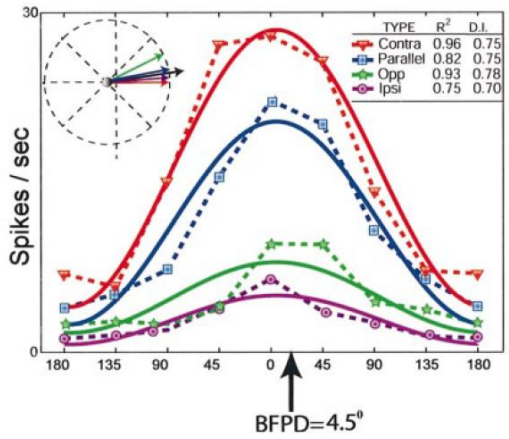


Fig 12: Best Fit Preferred Direction. Preferred direction of a neuron calculated for the four different movement types and as the BFPD **[17]**

In a second study, Donchin et al. attempted to understand the origin of bimanual related activity [16]. In particular they tested the hypothesis that bimanual activity results from a linear combination of unimanual activity related to each of the arms.

For this analysis they didn't directly use firing rate values but rather used the differences in firing changes with respect to the baseline firing frequency. For that they calculated the so-called normalized evoked activity (NEA) as follows:

$$NEA = \frac{(firing\ rate - baseline)}{baseline}$$

With that value they tested four different hypotheses:

1. bimanual NEA equals contralateral NEA
2. bimanual NEA equals ipsilateral NEA
3. bimanual NEA equals the sum of contra and ipsilateral NEA
4. bimanual NEA results from a linear combination of unimanual NEA

They falsified all of those hypotheses for the majority of the neurons showing a strong bimanual-related effect. These findings state that the differences in neural activity during bimanual and unimanual movements can't be justified by simple linear combinations of the two unilateral movements performed separately by each arm.

In a more recent paper the same group attempted to identify the origin of these variations, given that the linear hypothesis had proven to be wrong. They proposed a network mechanism by which the contralateral arm modifies the preferred direction and modulation depth of the ipsilateral arm through callosal projections. They called this the Mechanism of Callosal Inhibition [8].

Here is a summary of the major findings so far relating to how bimanual activity is encoded:

- Single neurons show directional tuning for unimanual movement
- There are considerable differences between bimanual and unimanual neuronal activity
- These differences cannot be explained by a linear combination of unimanual responses

2.4 Applications

Human motor behavior is extremely complex and many daily actions require the simultaneous use of the two arms. In order to develop prostheses and interfaces for people having some kind of neurologic problem it is crucial to understand how these movements are generated in the cortex. Due to this lack of understanding, only BMIs that allowed the movement of one arm at a time existed until recently.

Several approaches for the development of such interfaces have been taken. These range from displaying the arm position with a computer cursor to displaying it with a robotic arm or an avatar arm.

Carmena, Lebedev et al. developed in 2003 a BMI that allowed monkeys to reach and grasp virtual objects by moving a robotic arm (Fig 13) [18] [19]. Several parameters such as position, velocity and gripping force were extracted from neuronal activity of frontoparietal areas to accurately operate the robotic arm.

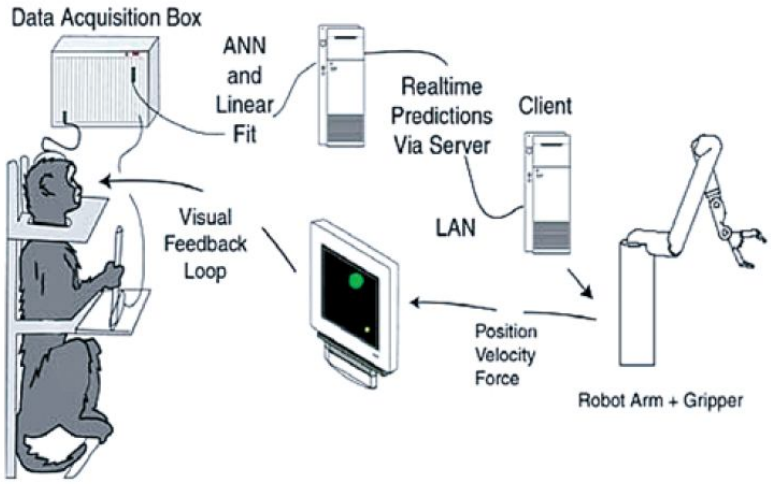


Fig 13: Schematic of the paradigm used for a unimanual BMI [18]

This and other experiments prepared the ground for the development of the first generation of brain controlled unimanual arm prosthesis for humans. A famous case was that of Cathy Hutchinson who in 2011 was able to lift a cup for the first time after 15 years of paralysis [20].

Then, a paper published in 2013 presented the first BMI that allowed monkeys to move two avatar arms simultaneously [9]. In this study neural activity was decoded using a filter that represented movement kinematics. The nonlinear relationships between neuronal rates and kinematic parameters were incorporated in the filter. With this study they showed that BMIs could be used for bimanual tasks in subjects that are unable to produce arm movements. The data of this study will be analyzed for the current project. This study and other advances in the field contributed to the first brain controlled bimanual arm prosthesis for humans. In December 2014, a group at Johns Hopkins University managed to have a double amputee perform a range of accurate motions [21].

2.5 Brain-Controlled Bimanual Experiment

The data analyzed in this project was collected at Duke Center for Neuroengineering in 2012. In this study, the first BMI was developed that enabled bimanual reaching with avatar arms. The first report of this work, *A Brain-Machine Interface Enables Bimanual Arm Movements in Monkeys* [9], explains how two monkeys were able to control bimanual movements using the BMI. Importantly, large-scale multi-area cortical recordings made it possible to simultaneously control two avatar arms.

2.5.1 Experiment Setup

Monkeys were implanted with multielectrode arrays in the cortex and the activity of the neurons was decoded with a decoding/training paradigm using a fifth-order unscented Kalman Filter (UKF). The decoding gave information about the intended position of the avatar arms and allowed to directly control them by cortical activity and independently move them towards two screen targets. Once the targets were reached, the monkeys were rewarded with fruit juice (Fig 14).

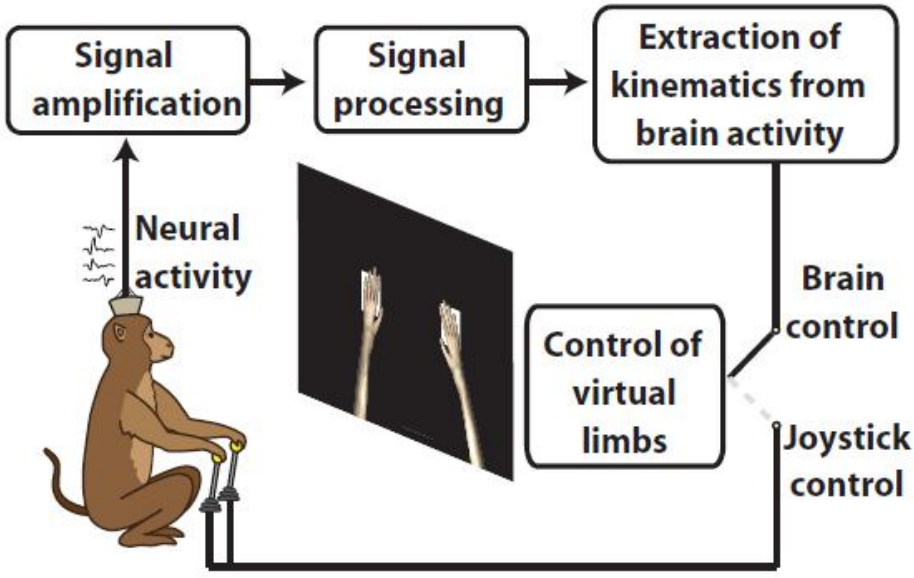


Fig 14: Setup for the bimanual task experiment performed at the Duke Center for Neuroengineering in 2012 [9]

2.5.2 Data Acquisition

The experiment was performed with two rhesus monkeys (M and C). Each of them had implanted electrode arrays, which recorded extracellular neuronal activity from up to 497 neurons from frontal and parietal areas of both hemispheres. The majority of the electrodes were placed in sensorimotor areas of the cortex (Annex 2). The electrode implant differed significantly between the two monkeys. Monkey C was implanted with eight 96-channel arrays, yielding a total of 768 microelectrodes (Fig 15). Each array was organized as 4 x 10 grids of shafts, with adjacent shafts spaced at 1mm. Monkey M was implanted with four 96-channel electrodes, a total of 384 implanted microelectrodes. In this case the arrays were composed of 4 x 4 grids with three electrodes per cannula.

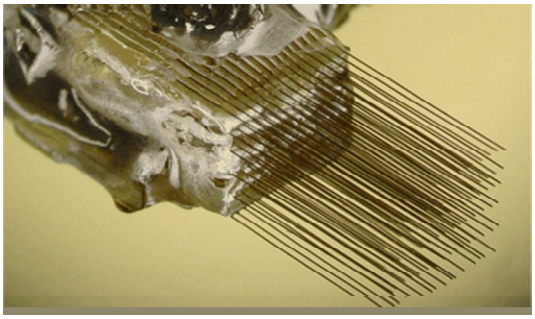


Fig 15: Implantable electrode array
(Duke Center for Neuroengineering)

2.5.3 Task Description

The objective of the behavioral task was to reach two targets with the two avatar arms simultaneously by controlling them with the brain through the BMI. For the experiment three different types of tasks were performed: joystick control, brain control with arm movements (BC with arms) and brain control without arm movements (BC without arms). In the joystick control tasks, the monkeys directly controlled the two avatar arms by moving the two joysticks. In BC tasks monkeys controlled the movement of the arms directly by their cortical activity. The two BC tasks differed with respect to the movements of the monkey's own arms. In BC tasks with arm movements the monkeys continued manipulating the joysticks and the arms were allowed to move freely even though the arm movements per se did not contribute to the control. In BC tasks without arm movements, the joysticks were removed from the apparatus and the arms were softly restrained to prevent overt movements (Table 1).

	Joystick	BC with arms	BC without arms
Control type	Joystick movement	Cortical Activity	Cortical Activity
Arm Movement	Allowed (required)	Allowed (disregarded)	Restricted

Table 1: Trial types in the bimanual experiment [9]

Monkeys learned through a different learning sequence how to perform BC movements without moving their arms (Fig 16).

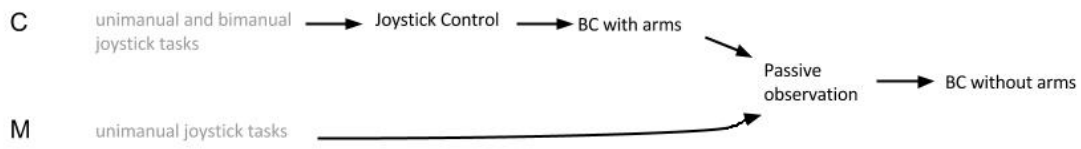


Fig 16: Learning process for both monkeys during the bimanual experiment

Before the experiment, monkey C was overtrained for about 12 months on both unimanual and bimanual center-out reaching tasks. Monkey C began by performing bimanual joystick control tasks until a high level of accuracy was reached (97% of the trials were correct). Following this, monkey C performed BC with arm tasks until the accuracy of the movements exceeded 75%. This level of accuracy was reached after 24 sessions within 7 weeks. For monkey M the procedure was somewhat different. Before the study monkey M was extensively trained on unimanual joystick tasks with the left arm, however during the study this monkey never used a joystick. Monkey M was not introduced to bimanual joystick tasks in order for him to be intentionally naïve to the bimanual BMI tasks before starting the passive observation experiments. At this level both monkeys participated in four experiments that were exclusively devoted to passively observing the two avatar arms move on the screen. In these experiments both arms were fully restrained. Right after the passive observation sessions both monkeys were engaged in BC without arms sessions. Monkey C and Monkey M needed 9 and 15 sessions respectively to reach proficiency.

The structure of the experiment during two recording sessions was as follows: 150 bimanual trials, then 150 unimanual left trials, followed by 150 unimanual right and finally 100 to 200 extra bimanual trials if the monkey was still cooperative. The objective of the bimanual trials was to reach with both hands to their respective target. Targets were presented in four possible positions for each hand (top, bottom, left and right) on a circle with a fixed radius of 8 cm centered at the center target or rest position. Due to the simultaneous reaching of the targets for both arms, there were a total of 16 potential target combinations for bimanual tasks. In both unimanual and bimanual tasks the target combinations were assigned randomly and were equally likely.

A trial began in the central position for the avatar hands, signaled with two square targets in the screen. The monkey had to move the avatar arms to those positions and hold them there for an interval of time that varied randomly from 400 to 1000 ms. Once the avatar arms were in the start position, the central position squares were replaced with two circular targets at

one of the possible combinations. The monkey had to simultaneously move the avatar arms to their respective targets and hold them there for a minimum of 100 ms (Fig 17). After this delay a juice reward was given to them. In the unimanual version, only one avatar arm was displayed and had to reach a single target.

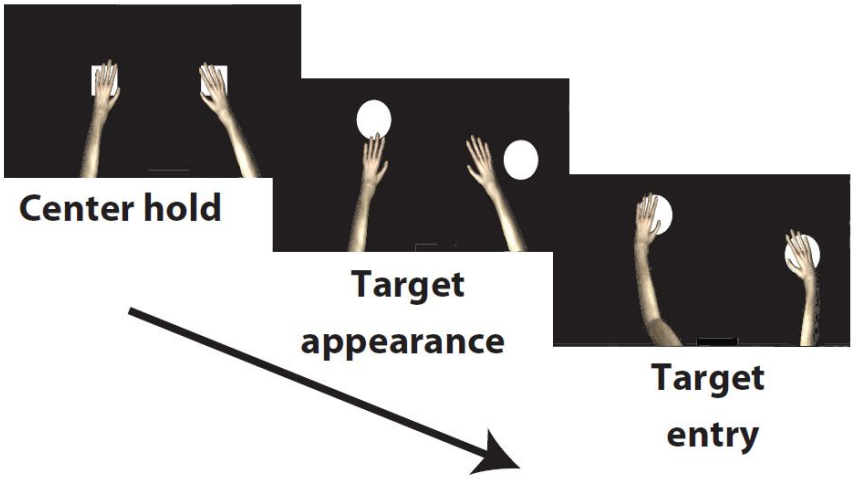


Fig 17: Trial Sequence [9]

2.5.4 Decoding Algorithm

Many real-time decoding methods in BMI studies have utilized linear models of neural tuning. These models describe the relationship between neural activity and limb movement as linear. These models stemmed from Georgopoulos' observations of the cosine-shaped directional tuning of neurons in the cortex and its resultant population vector hypothesis. In this hypothesis the movement direction is calculated by summing individual neuron vectors pointing in their preferred directions and scaled by their firing rates. Many BMI studies were performed using this approach. Additionally several other filters were developed that improved accuracy when compared to the population vector approach. The Wiener filter, an optimal linear regression method, became popular and is still widely used because of its simplicity and efficacy [22]. Then the Kalman filter caught attention as research on new decoding algorithms progressed [23].

The main innovation of the Kalman filter was the explicit separation between the model of the relationship between neural activity and movements and the model of how movements progress with time. In other words, the Kalman filter uses measurements over a period of time to produce variable estimates. This algorithm tends to be more precise than others based on

single measurements. The process consists of two steps. First the filter estimates current states variables. Then, with the information of the new measurement outcome, the variable estimates are updated in a way that gives more importance to the estimates with higher certainty. This algorithm runs in real-time and needs only the current measurements and the last calculated state as inputs. However linear models are limited since they don't exploit the useful, abundant and available statistical information contained in the neural data. For this reason non-linear models, although more complex, have proven to describe neuronal modulations better than linear models.

The unscented Kalman filter is a non-linear variation of the standard Kalman filter. The advantage of such a filter is that it achieves non-linear filter improvements without the need to use a particle filter, the standard filter designed for non-linear observation models that is computationally heavy [24]. Within unscented Kalman filter, the higher the order of the filter, the higher the number of recent states that will be used to estimate the variables. This highly improves the accuracy of the movement prediction.

The kinematics of both arms were therefore decoded from cortical activity using a fifth-order Unscented Kalman Filter (UKF). For Monkey C, the model was first trained using 5 to 7 minute data of joystick control trials. Thereafter the model was used and fit in BC with arms trials where the avatar arms were already under the control of the decoder. In each session the UKF was retrained for 5 to 7 minutes during the passive observation trials. The passive observation was the base for the BMI training, without the need to have the monkeys perform overt arm movements. After such training the avatar was controlled by the decoding algorithm.

For offline analysis, unimanual trials' neural activity could be fit by the tuning model in terms of the arm position, velocity and their quadratic terms:

$$y(t) = [b_1\ b_2]\begin{bmatrix} pos_x(t) \\ pos_y(t) \end{bmatrix} + b_3\sqrt{pos_x(t)^2 + pos_y(t)^2} + [b_4\ b_5]\begin{bmatrix} vel_x(t) \\ vel_y(t) \end{bmatrix} + b_6\sqrt{vel_x(t)^2 + vel_y(t)^2}$$

Bimanual activity could be fit in a similar way using information about both arms in this case.

$$y(t) = [b_1\ b_2\ b_3\ b_4]\begin{bmatrix} pos_{xL}(t) \\ pos_{yL}(t) \\ pos_{xR}(t) \\ pos_{yR}(t) \end{bmatrix} + [b_5\ b_6]\begin{bmatrix} \sqrt{pos_{xL}(t)^2 + pos_{yL}(t)^2} \\ \sqrt{pos_{xR}(t)^2 + pos_{yR}(t)^2} \end{bmatrix} +$$

$$[b_7\ b_8\ b_9\ b_{10}]\begin{bmatrix} vel_{xL}(t) \\ vel_{yL}(t) \\ vel_{xR}(t) \\ vel_{yR}(t) \end{bmatrix} \quad + \quad [b_{11}\ b_{12}]\begin{bmatrix} \sqrt{vel_{xL}(t)^2 + vel_{yL}(t)^2} \\ \sqrt{vel_{xR}(t)^2 + vel_{yR}(t)^2} \end{bmatrix}$$

The UFK therefore allows to independently control both arms despite jointly representing them in a single decoding paradigm.

3 Materials and Methods

3.1 Bimanual Experiment Data

The data analyzed in this Thesis was acquired in 2012 at the Duke Center of Neuroengineering during the bimanual BMI experiment described in the previous section [9]. In this study, researchers developed the first bimanual BMI.

The experiment examined an arm reaching task. Monkeys were shown targets on a computer screen, and their movements of two joysticks were shown as movements of two avatar arms displayed on the same screen. Depending on the type of movement (unimanual vs bimanual) they had to acquire either one or two targets simultaneously. Each of the targets was randomly assigned to one out of four possible locations (right, left, top, bottom). For bimanual movements this corresponded to a total of sixteen possible combinations (Fig 18).

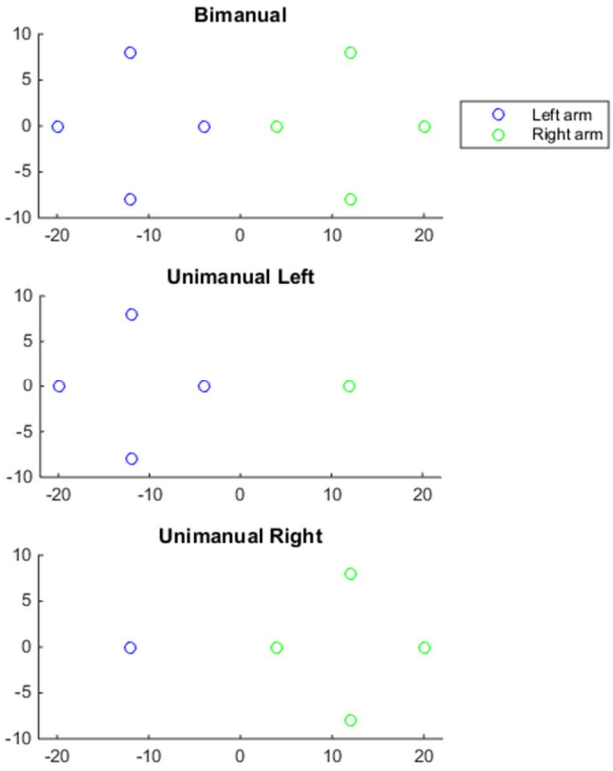


Fig 18: Target positions. Possible target positions for unimanual and bimanual trials. Units are given in cm

We can distinguish between three different types of bimanual movements: parallel, opposite and complex. Parallel movements are movements where the arms move in a parallel fashion since targets are located in the same position for both arms. In opposite trials, the arms perform movements in opposite directions. Finally complex movements correspond to all other movements where the difference in direction between the arms is 90 degrees. There are four possible target configurations for parallel movements, four for opposite movements, and eight for complex movements.

Because the electrodes of Monkey C were located at many different positions in the brain and thus gave additional information to analyze, all the data used in this project correspond to Monkey C. Before learning the BMI control, this monkey was trained to perform the reaching task with two joysticks. In this Thesis we were interested in studying the representation of bimanual arm movements in the cortex. Therefore, we only analyzed the recordings of the joystick control where the monkeys performed overt arm movements.

The data used for the study were acquired on three different days: two of them correspond to bimanual trials and two of them to unimanual left or right trials. We selected only the correctly performed and rewarded trials for the present analysis (Table 2).

	Bimanual	Unimanual Left	Unimanual Right
Number of valid trials	713	420	306
Total number of trials	1061	711	469

Table 2: Number of available trails Number of trails available for the study for each movement type

Plots of the trajectories of the monkey's arm during the trials can be found in the Annex (Annex 3). These plots illustrate movement accuracy for different target locations. Note that the trajectories for bimanual movements are far less accurate than those for unimanual movements.

3.2 Data Analysis

3.2.1 Data Format

The data from the experiments were organized in different files. All data from a given recording session were stored in a folder corresponding to that day. Then the folder contained a text file specifying the type of session (unimanual right, unimanual left or bimanual), raw spiking data, binned neural data and other non-neural parameters concerning the trials. Raw spiking data is presented in the form of vectors, one for each neuron. Each matrix is labeled with the specific neuron identifier and indicates the times at which that neuron was detected to be firing. This information was processed and stored in a larger matrix containing the spiking information of all the neurons during a given session. In order to integrate the information of all neurons, neural data had to be binned. This technique allows to group spikes in temporal bins. In particular the bins used in this project were 10 ms bins. In this way if a neuron spikes at t_1=34.035 s and at t_2=34.039 s, since both events are within the same bin going from 34.030 s to 34.040 s, that bin will have 2 as a value (Fig 19).

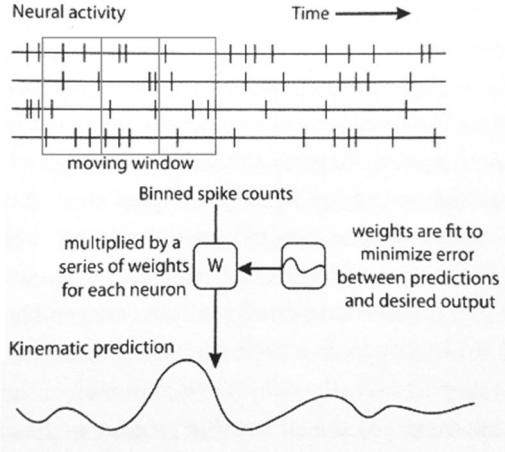


Fig 19: Translating raw neuronal activity into digital commands [29]

Although it is true that this technique causes a loss in temporal resolution, it does not affect posterior analysis that will be performed using larger time windows. Furthermore it facilitates a lot the analysis of the data since the spiking of several neurons at a specific moment (specific bin) can be directly compared. Such a straightforward comparison is not possible with raw data because of the discrete nature of the data and the fact that it is extremely unlikely that two neurons fire at the exact same millisecond. This pre-processing was done beforehand and the data used in this project directly corresponds to the binned matrices. The input neural data thus corresponds to several m x n matrices containing neuronal firing rates where m is the number of bins and n is the number of neurons. The total duration of each recording session is of m*(10 ms) = m/100 s.

Some additional information was required for the study. In particular a "trial" structure containing many details of the experiment was included for each recording day. This structure contains one entry per trial, which gives an average length of around 500 for each day. There are many different fields giving specific information about each trial. The following parameters are those more significant for the current study. In a first place detailed temporal information is given concerning: target presentation, movement onset, target reach, movement stop, reward or return to central position. In the same way it is specified for each trial whether the movement has been successful and a reward has been given, or the movement was not successful due to time excess or monkey inattention. The type of control was also specified: hand control, brain control with arm movements or brain control with arm movements restricted. Importantly the target positions of each trial were determined as coordinates for each of the two targets. This piece of information will be central to study later how neural

activity correlates with the movement direction. Finally, data detailing the instantaneous position of the joystick as well as the movement velocity was included.

3.2.2 Data Filtering and Smoothing

Recorded neural data are highly noisy. This is due to several reasons. First of all, neurons fire at relatively low rates so 10 ms binned data consist mostly of zeros with occasional ones and rarely twos. Second, the neural coding in the brain has a stochastic nature, which causes the recorded signal to be noisy and have high inter-trial variability (Fig 20).

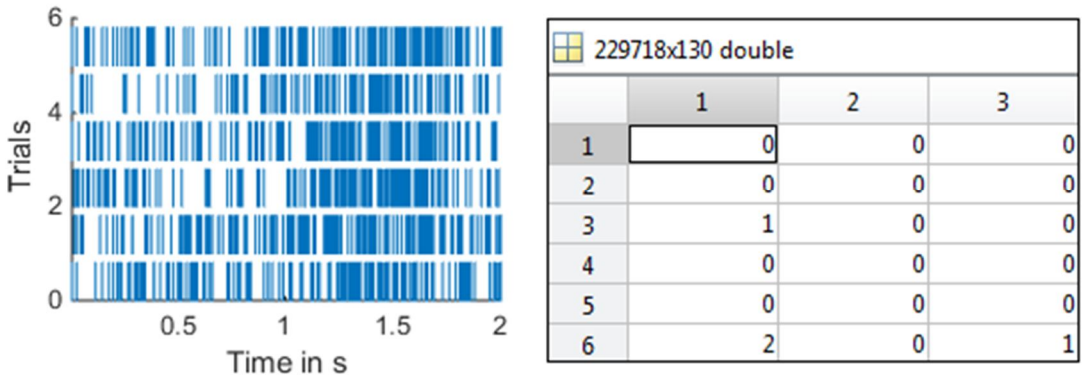


Fig 20: Raw spike trains and digital commands. Spike trains for a given neuron during six different trials in a 2s period (movement onset happens at t=1) and an example of neural information as it looks in the binned matrix

As we can see in **Fig 20** it is not easy to detect a behavioral signal of interest when directly looking at neural activity during an individual trial or even during several trials. A preliminary study was done in order to verify that a neuronal response was visible and clearly attributable to movement. For that raw spike trains during movement together with a smooth curve obtained from several trials were plotted (Fig 21). This smoothing was done using a Gaussian kernel. The Gaussian kernel is one of the most commonly used kernels and it has been widely used in neural data smoothing [25]. The kernel is given by:

$$GK(x) = \frac{1}{\sqrt{2\pi}\sigma}\exp\left(\frac{-x^2}{2\sigma^2}\right)$$

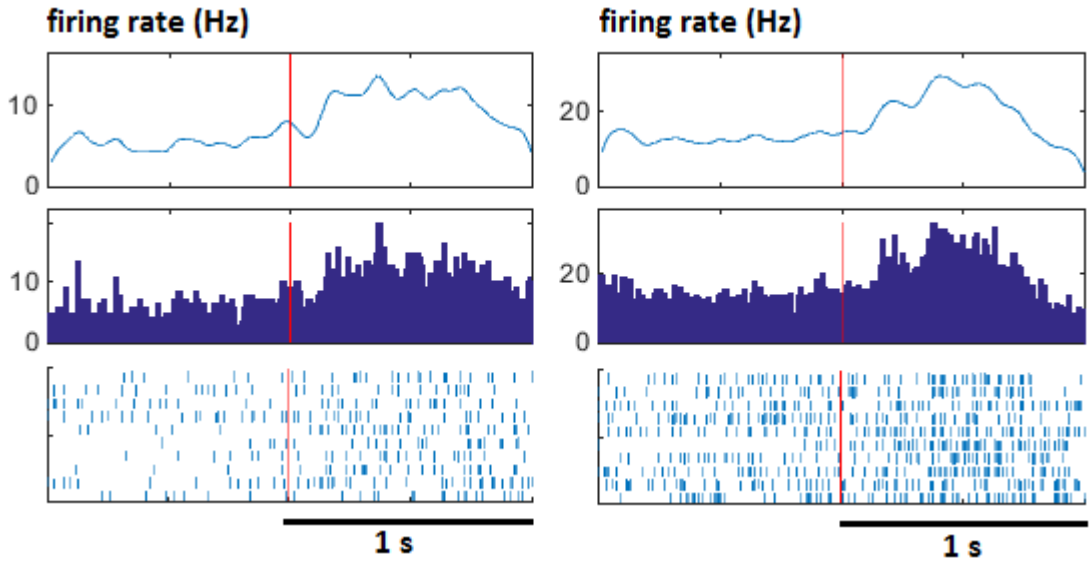


Fig 21: Smoothed neural activity. Smoothed activity, PETHs and raw spike trains of two neurons during ten trials in a 2s period. The upper curve is obtained after smoothing all trials with a 300ms Gaussian kernel. The red line shows the moment of movement onset

The three curves in **Fig 21** show a clear increase in neuronal activity right after the movement onset. These results were not easy to detect from the spike trains only. This pre-processing was performed to ensure the validity and usefulness of the data for the study.

3.3 Models of Neuronal Directional Tuning Properties

The first step in the analysis on the relationship between neuronal activity and movement was to characterize the neuronal response for each movement. This was done in the following way. The response of a given neuron was characterized as firing frequency per second. For a given neuron and a given trial, only the spikes occurring in a time window going from 250 ms before movement onset to 1000 ms after movement onset were considered. This time window is selected because the 250 ms prior to movement capture preparatory activity for the movement and the 1000 ms posterior to movement onset capture movement. In this way if a neuron fires 25 times in that period, the firing frequency will be f = 25 / 1.25 = 20 spikes/s. Therefore the response of a given neuron for a given trial was determined by a scalar: the firing frequency. It is important to note that from here on, most of the tuning analysis considers firing rates averaged over these time windows and does not focus on time dependent changes of firing rate within the windows.

Several approaches were employed to extract tuning information from this data. One goal of the project was to compare these different methods and study their reliability. I started with the cosine fitting approach that is a standard in this kind of studies. This approach is applicable to the analysis of tuning curves, i.e. responses obtained for a given neuron by averaging firing rates for all trials with the same target configuration. I then tested a different approach where no averaging across trials was done prior to the regression analysis.

3.3.1 Standard Cosine-Fit Model

So far, the standard approach that has been used to characterize tuning properties of neurons is the cosine-fit model first introduced by Georgopoulos in 1982. His results suggest that neurons that are directionally tuned to movement exhibit a cosine-shaped response to the movement direction. That is, their firing frequency has a maximum value when the movement is performed in their preferred direction and this firing frequency decays as the movement direction shifts away from that preferred direction, falling to a minimum value in the direction opposite to the preferred one [3]. Based on that observation, the common approach to characterize tuned neurons has been to fit the firing frequencies obtained for the different movement directions to a cosine curve. The better the frequencies fit the cosine, the more specifically that neuron is considered to respond to movement. These neurons are called directionally tuned neurons.

I performed this fit in the following way. Once I evaluated the response of each neuron for each trial as its firing frequency, I grouped together the response of each neuron for every target position. For the case of unimanual movements, the responses of a given neuron for all movements to the right were grouped together and averaged. The same was done for each of the other three movement directions. In a case where we have 100 trials equally distributed amongst the 4 targets (top, bottom, right and left) this means that the 25 responses corresponding to each of the targets are averaged to obtain only four characteristic frequencies. For bimanual movements, this is equivalent to grouping together all responses measured for a given combination of target locations. So for example, all responses of a neuron during simultaneous movement to the right of the right arm and to the left of the left arm were grouped together and averaged. The same was done for the rest of the sixteen possible target combinations. The input for the actual fit were therefore 4 data points for unimanual movements and 16 data points for bimanual movements.

The equation used for the fitting of the firing frequency of unimanual movements is:

$$firingFreq(\theta) = a * \cos(\theta - \theta_{PD}) + b$$

Where θ is the angle of the movement direction dictated by the target location, *a* is the amplitude of the response, θ_{PD} is the preferred direction given in radians and b is the baseline value of the activity. It is worth noting that *a* does not correspond to the amplitude of the spiking response of a neuron since this amplitude is invariant for action potentials in cells. The amplitude corresponds to the range of the binned activity during the chosen time window.

For the case of bimanual movements the equation turns on to be:

$$firingFreq(\theta_R, \theta_L) = a_R * \cos(\theta_R - \theta_{PDR}) + a_L * \cos(\theta_L - \theta_{PDL}) + b$$

In this case a_R, θ_{PDR}, a_L and θ_{PDL}, are the amplitude and preferred direction for the right and left arms respectively and b is the baseline activity of the neuron. θ_R and θ_L represent the angle at which the right and left targets are located with respect to the central rest position. The fits were calculated using the function *fit* of MATLAB and the four or sixteen data points, for unimanual and bimanual movements respectively, as input values for the fit. The code can be found in the Annex.

The following diagram shows the convention used for angle value as a function of movement direction of an individual arm for the whole project (Fig 22):

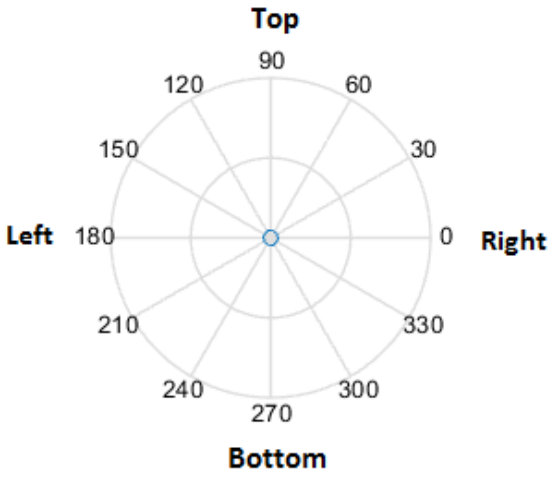


Fig 22: Reference of movement directions used throughout the project

Other than the characteristic values of amplitude, preferred direction and baseline, the function also gives as output the coefficient of determination. The coefficient of determination (R^2) quantitatively assesses the quality of the fit of the neuron activation response to a cosine

function. In order to be able to compare our results to previous results I have used the same criterion to define directionally tuned neurons that was used by Georgopoulos in 1982. He selected a value of 0.7 as a threshold to determine a neuron directionally tuned and this value has often been used since then as a reference.

For comparison purposes with previous studies performed at the Hebrew University by Steinberg et al. [8] [17] some supplementary fits have been done for particular bimanual movements. In their papers the authors only study certain combinations of bimanual movements, in particular opposite and parallel movements. As a reminder, parallel movements are movements where the target is at the same location for both arms (i.e. right and right) and opposite movements are movements where the targets are in opposite locations for the two arms (i.e. top and bottom). There are in total four possible parallel movements and four possible opposite movements and thus these fits are also performed with only four data points each.

Finally some adjustments had to be done to obtain the true angle values for the preferred directions. In particular the principal value of the polar angle had to be obtained for further analysis: for that all angles were translated into angles ranging from $-\pi$ to $+\pi$. A second adjustment had to be done since the fit would sometimes give negative output values for the amplitude and therefore the preferred direction angle would be shifted by 180 degrees or π radians.

3.3.2 General Regression Model

Based on findings of the previous section and on principles of neurophysiology it was important to evaluate an additional approach. As we have already seen, neurons are highly noisy. However noise is an important property and should not be neglected. In particular we believe that the higher the relative noise of a spiking neuron when a particular action is being done, the lower its correlation and significance for that action is. Alternatively, if a neuron has a high signal-to-noise ratio during a given action, then probably its contribution will be relatively important to conduct the action. For this reason, characterization of neuronal responses as signal-to-noise ratio is important for neurophysiology and BMIs. In particular, BMIs strive to increase signal-to-noise ratio by ensemble averaging for a population of neurons. Additionally, using average tuning curves without the calculation of the signal-to-noise ratio is a common error found in many papers [26], which misrepresents poorly tuned

neurons as significant. Note that averaging across trials (see previous sections) cancels out noise, which is good for illustrative purposes, but eliminates an important characteristic of neuronal response. Therefore the traditional cosine-fit method that I presented above is good to assess the shape of the tuning curve, but does not provide enough information on the relationship of a given neuron to action.

For this reason, the model of signal-to-noise ratio should be based on the following principles:

- No across trial averaging is done to preserve neuronal noise
- Regression analysis is applied to trial data instead of the average tuning curve

Based on these two premises we propose a multiple linear regression model of directional tuning where the tuning characteristics are extracted from the trial data for a given cell, without performing any averaging prior to the regression. To illustrate, for the case of unimanual movements this corresponds to performing the regression with 300 to 400 data points, instead of fitting with only four averages. Each of these data points is a function of the firing frequency measured during the chosen time window for a single trial. In particular each data point is calculated as the firing frequency of a neuron during a specified time window of the trial divided by the standard deviation of that neuron during that same window across all trials.

$$neuronalResponse = \frac{firingFreq\ during\ an\ individual\ trial}{standard\ deviation\ across\ all\ trials}$$

This normalization, which basically expresses data as a signal-to-noise ratio, removes unwanted biases when characterizing the response strength, such as a bias toward cells with high firing rates. Cells with high firing rates may appear strongly tuned, but actually they could be very noisy. Normalizing by the standard deviation removes this problem because, again, responses become expressed as a signal-to-noise ratio. In my analysis, this regression was conducted independently for each movement type (unimanual right, unimanual left and bimanual).

The equation used for unimanual movement multiple linear regression is given by

$$firingFreq(x, y) = a * x + b * y + c$$

x and y being the coordinates of the target location and a, b and c the coefficients of the regression. Here { a , b } is the vector pointing in the preferred direction of the neuron. Since

the equation corresponds to a scalar multiplication of { a , b } by { x , y }, the firing frequency of the neuron reaches a maximum value when { x , y } points in the same direction as { a , b }.

For bimanual movements the multiple linear regression equation is:

$$firingFreq(x_R, y_L, x_R, y_L) = a_R * x_R + b_R * y_R + a_L * x_L + b_L * y_L + c$$

where x_R,y_R and $x_L, y_L$are the target locations for the right and left hands respectively and a_R, b_R, a_L, b_L and c are the regression coefficients.

This method allows us to model the relationship existing between the dependent variable and the independent variables (explanatory variables or predictors) by estimating the weight or contribution of each explanatory variable to the dependent variable.

In order to perform a multiple linear regression on the data, the MATLAB function *regress* was used:

$$b = regress\ (Y, X)$$

This function receives as inputs an n x 1 vector Y of responses and an n x p matrix X of predictors and returns as output a p x 1 vector b of coefficient estimates, where n is the number of observations and p is the number of predictors (see Annex for details). For our purposes n, the number of observations, corresponds to the number of trials studied whereas p, the number of predictors, corresponds to the number of coordinates of the positions of the different targets used (2 or 4 depending on the movement type). X, the predictor matrix, has the target location coordinates for each trial (*x* and *y* positions for one or two targets). Y is the n x 1 vector of neuronal responses expressed as signal-to-noise ratio where n is the number of observations or individual trials.

To compare the approaches with or without previous averaging of the trials we calculated the similarities between the preferred directions obtained for each of them. Similarity was calculated as correlation between the values.

Finally, based on previous observations relating to neuronal noise, we decided to characterize neuronal tuning as tuning depth instead of classifying neurons according to the coefficient of determination. Tuning depth, which corresponds to the amplitude of the regression once the firing rates have been divided by the standard deviation of the cell, is a signal-to-noise ratio whose value reflects the relationship of neuronal rate to movement kinematics, given a linearity assumption. (For better fitting, nonlinear models could be applied, but the linear

model approximates the data reasonably well in our assessment). In order to classify neurons as tuned or non-tuned we have to define a threshold value above which neurons are considered as directionally tuned. Since such classification cutoff is usually selected arbitrarily, I chose an approach that better described statistical significance for my dataset. I selected the amplitude threshold as the one corresponding to a p-value of 0.05. As shown in the next section, this corresponded to an amplitude value of 0.2. Accordingly, this amplitude value was applied universally to classify neurons as directionally tuned.

Furthermore I analyzed what would happen if the classification of neurons was done using the coefficients of determination for the cosine-fit of the trial averaged data. I discovered that such an approach could lead to misleading outcomes. In this analysis, I calculated for each neuron its relative position within the neuronal population when directional tuning is concerned, ranging from non-tuned neurons to extremely well-tuned neurons. I divided the population in twenty quantiles and studied for each neuron how much this change in definition for directional tuning affected its position within the population. This was measured as the number of quantiles by which the neuron was displaced when applying one or the other definition. I qualitatively assessed the accuracy of this alternative definition by observing the specific responses of neurons for which the relative position within the population varied by many quantiles.

3.4 Analysis of Movement Representation in Neurons

The major goal of this project was to study differences in directional tuning for the different movement types. In particular we were interested in assessing whether the percentage of neurons that were tuned to movement varied for the different movement types. The tuning properties are the amplitude (tuning depth), baseline and preferred direction. All these were obtained by doing the general multiple linear regression presented above, where the individual responses of each neuron for each trial are divided by the standard deviation of the neuron and then regressed without any averaging against the coordinates of target location. Specifically tuned neurons were defined to have tuning depths larger than 0.2.

3.4.1 Tuning Degree of the Population

As a first study of the parameters obtained with the regression I compared the tuning degree of the population for different types of movements. The tuning degree of the population refers to the percentage of neurons that exhibit directional tuning during a given movement type. In a first place I calculated the tuning percentage in unimanual movements in terms of the position of the neuron with respect to the arm, ipsilaterality and contralateraly, instead of considering the absolute side of the arm in the body. For a given neuron a movement is ipsilateral if it is performed with the arm located in the same side of the body (neuron of the left hemisphere when the left arm is moved) and contralateral if it is performed with the opposite side of the body (neuron in the left hemisphere when the right arm is moved). Neuronal tuning was therefore studied separately for unimanual movements of the right and left arms in order to obtain specific information about responses to ipsilateral and contralateral movements. Furthermore in order to compare our bimanual movement results with previous literature, the tuning for bimanual opposite, parallel and complex movements was also analyzed. As a reminder, complex movements are defined as movements requiring more coordination that opposite and parallel movements, which correspond to all the other possible combinations and in which the target angle difference for both arms is 90 degrees. To study the tuning of those movements I performed a regression where I only included trials corresponding to each type of movement. For example, just 4 points were used for the parallel model whereas the regression for bimanual complex movements was done with the 8 possible combinations.

3.4.2 Preferred Directions Analysis

Next, I studied the preferred directions obtained for each type of movement. Again, the preferred direction of a neuron is defined as the movement direction for which a cell shows its strongest response and it is obtained with the multiple linear regression. For unimanual movements we only deal with one preferred direction whereas for bimanual movements we obtain two preferred directions (one for each arm). The fact that neurons respond more strongly to a specific direction in unimanual movements is widely known. However such behavior is not so readily observable for bimanual movements. In order to better understand how bimanual movements are represented in the brain and how that representation relates to the unimanual case, it is important to determine whether the preferred direction of a cell is

maintained for the two arms and for both the unimanual and bimanual cases. This idea can be summarized in two questions. Does a cell have the same preferred direction for the two arms? Does a cell maintain the preferred direction it exhibited in unimanual movements also during bimanual movements?

To elucidate cortical encoding of contralateral and ipsilateral arm movements, I will investigate the relationship between the preferred direction of a given cell for the left and right arms. The easiest way to analyze this is to calculate the difference in preferred direction between the two arms. The preferred direction is presented as angles and thus the difference corresponds to an angle value ranging from 0 to 180°, since 180 is the maximum possible angular distance between two points. I will plot the distribution of this value distinguishing between unimanual and bimanual movements to study the possible differences. The relationship obtained for each movement mode (unimanual/bimanual) will be calculated as the correlation.

Secondly I will analyze if the preferred direction for a given arm is maintained from unimanual to bimanual movements. That is, if the preferred direction for a given neuron is Θ in unimanual movements, is the preferred direction in bimanual movements Θ too? To study this I will proceed in the same manner as above and calculate the differences between the angles found in each of the cases. I will also scatter the results and calculate the correlation. Furthermore the differences in preferred directions for the ipsilateral and contralateral arms will be studied separately.

3.4.3 Linear Hypothesis Testing

One of the objectives of the project was to test the linear hypothesis for cortical representation of bimanual movements. The linear hypothesis states that the cortical response to bimanual movements stems from a linear combination of the cortical activity during separated unimanual movements. This would mean for example, that the activity recorded when the two arms are moved simultaneously to the right should be a linear combination of the independent unimanual movements of the right arm to the right and of the left arm to the right, the simplest linear combination being the sum of two responses. Mathematically, this means that if the response of the neuron is given by:

Right arm: $firingFreq(\theta_R) = a_R * \cos(\theta_R - \theta_{PDR}) + b_R$

Left arm: $firingFreq(\theta_L) = a_L * \cos(\theta_L - \theta_{PDL}) + b_L$

Then the bimanual response will be given by

$$firingFreq(\theta_R, \theta_L) = k_R * (a_R * \cos(\theta_R - \theta_{PDR}) + b_R) + k_L * (a_L * \cos(\theta_L - \theta_{PDL}) + b_L)$$

Where k_R and k_L are the weights given to each arm. Importantly the individual amplitude, preferred direction and baseline are maintained when calculating the response for bimanual movements.

Based on the study performed in a similar paper I analyzed this idea by studying four different hypotheses [16]:

- Bimanual responses equal contralateral unimanual responses
- Bimanual responses equal ipsilateral unimanual responses
- Bimanual responses are equal to the sum of contralateral and ipsilateral responses
- Bimanual responses arise as a linear combination of unimanual responses

For a given hypothesis I simulated what the neuronal response to bimanual movements would have been based on the unimanual data I had, assuming the hypothesis to be true. The simulation was straightforward in the three first cases. For the last hypothesis, I performed a regression using the unimanual right and unimanual left tuning parameters (preferred direction, amplitude and baseline) as well as the actual bimanual response. Once I had the coefficients of the regression, these were multiplied by the unimanual responses in order to get a simulated weighted response. Finally I calculated for each neuron and each hypothesis the correlation between the simulated response and the actual recorded response and studied the distribution of correlations amongst the neuronal population.

3.4.4 Movement Complexity

I also studied the effects of movement complexity on the tuning properties of neurons. Movement complexity specifically refers to the difficulty associated with the different types of bimanual movements. It is well known that opposite and parallel movements are easier to perform compared to complex bimanual movements. Complex movements are harder to perform because there is no simple relationship between the actions carried on by each arm and therefore it is harder to perform such different movements simultaneously. We wanted to see whether this has an effect on the tuning degree. First, I studied whether the percentage of

tuned neurons varies with the type of movement. Second, I studied whether some movement types tend to have higher shifts of the preferred direction than others. This was studied in a similar way as explained above. Finally I tested the linear hypothesis in the different movement types to determine whether it holds better for some of them. All information derived from this study is crucial to infer how bimanual movements are represented in the brain.

4 Results

4.1 Linear Models of Neuronal Directional Tuning Properties

The first part of the project includes several approaches to model the response of cortical cells as a function of movement direction. First, I will present the results obtained when performing the standard cosine-fit that has been widely been used in previous literature. Second, I will present the results obtained with the regression model developed for the project where no across-trial averaging is performed.

4.1.1 Standard Cosine-Fit Model

In a first place I performed a fitting using the standard cosine-fit approach with previous averaging of the data. That is, I averaged all the responses obtained for a given target position and defined in this way 4 (unimanual) or 16 (bimanual) characteristic values of neuronal response that were then used as input values for the cosine-fit (Fig 23) (Fig 24). This approach is very useful in order to display the behavior of a given neuron.

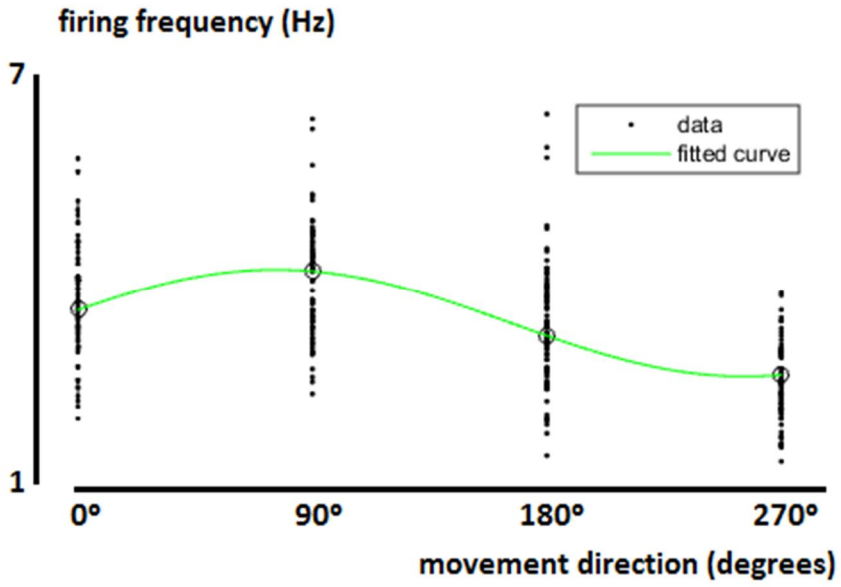


Fig 23: Variability in Neural Responses. Firing frequency of a neuron in several trials for different movement directions. Black dots represent individual trials and black circles represent average response for a given direction. The green line shows the cosine-fit of the averages.

Fig 23 shows a noticeable difference in firing frequency response of a given neuron as a function of the movement direction. Importantly we can see that the responses are very variable from trial to trial.

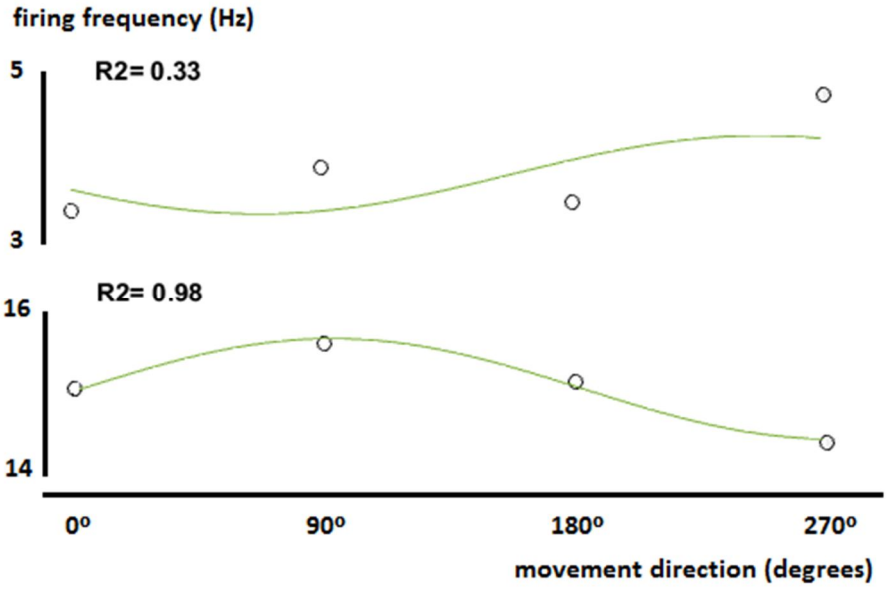


Fig 24: Cosine-fit. Cosine-fit of neuronal tuning for a non-tuned neuron and a highly tuned neuron. The x-axis represents the angle of movement and the y-axis represents the firing frequency of the neuron during movement. The black dots show the averaged firing frequency for each angle and the green curve is obtained by fitting them.

This example illustrates the fact that neurons whose activity matches well the shape of a sinusoid have a high coefficient of determination (R^2): the cosine fit is highly accurate in these cases and the neurons are considered to be directionally tuned. On the other hand neurons whose activity patterns cannot be well approximated by a sinusoid have a lower R^2 even if they appear directionally tuned on visual inspection. In particular the threshold to satisfy directional tuning was chosen to be a minimum of 0.7 for the coefficient of determination. When plotting the PETHs for tuned and non-tuned neurons, some clear differences can be identified (Fig 25).

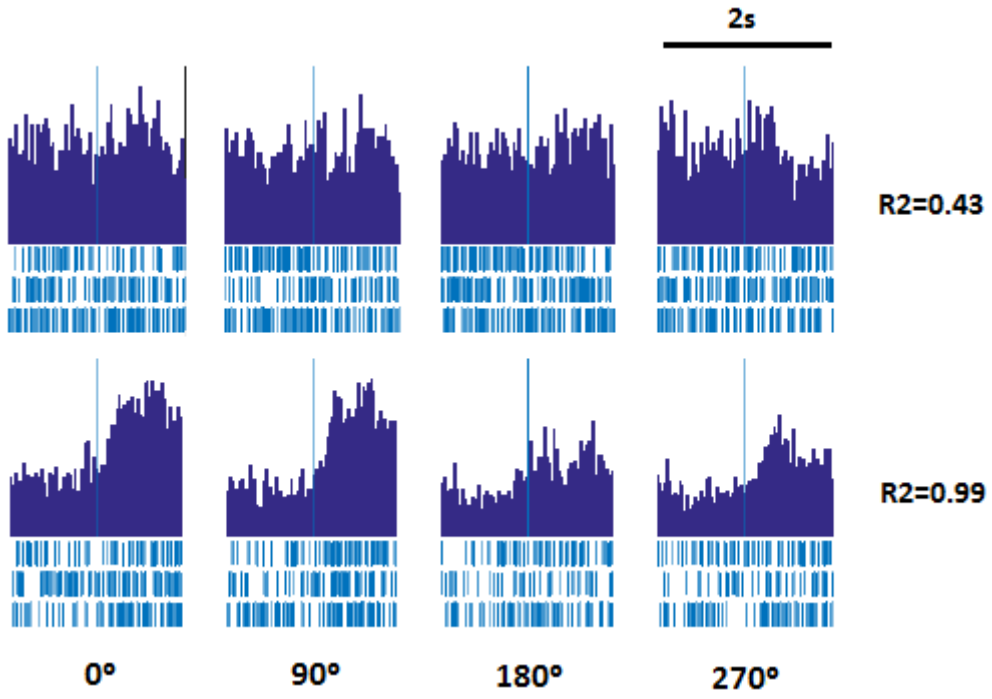


Fig 25: PETHs and spike trains for tuned and non-tuned neurons. Activity of two neurons considered to be or not directionally tuned for movements at different angles. The vertical blue line indicates movement onset

We can see that directionally tuned neurons have strong responses following movement onset and show preference to one of the directions where they exhibit higher activation rates. On the opposite non-tuned neurons do not show any clear response to movement onset and don't seem to respond more to any particular direction.

Even if this is the general trend, we also found some cases where the coefficient of determination did not seem to be indicative of the tuning properties of the neuron (Fig 26).

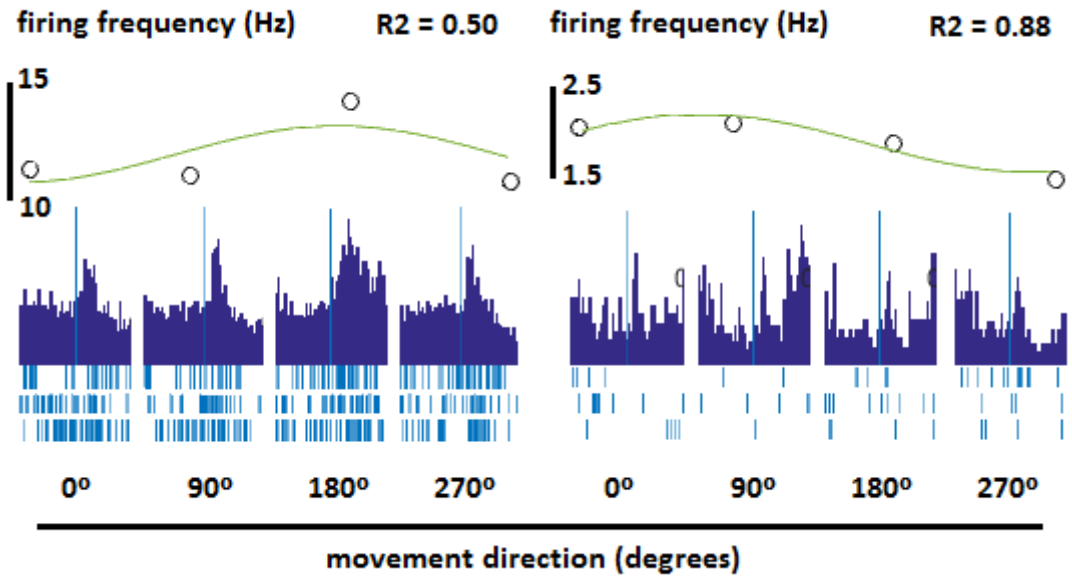


Fig 26: Cosine-Fit approach flaws. Cases of neurons where the coefficient of determination is not representative of their tuning properties

In the case of the first neuron one can see that the coefficient of determination R^2 is below 0.7, which is the criterion used to define a neuron as non-tuned. However we can visually identify a preferred direction, which is around 180 degrees. For the second neuron we can see that it exhibits PETHs with no clear directionally tuned response, despite having a very high coefficient of determination.

4.1.2 Regression Model without Previous Averaging

After having obtained the results by means of a model with previous averaging of the data for each target position, I studied the results obtained when doing this fit of the data without previous average. That is I performed the fit with all available data points for all target positions to perform the fit. In particular I present here the results obtained when performing a regression with all individual trials.

I compared the tuning parameters obtained with this method to those obtained using the standard cosine-fit approach. First, I compared the preferred directions obtained for the neurons in both unimanual and bimanual movements (Fig 27).

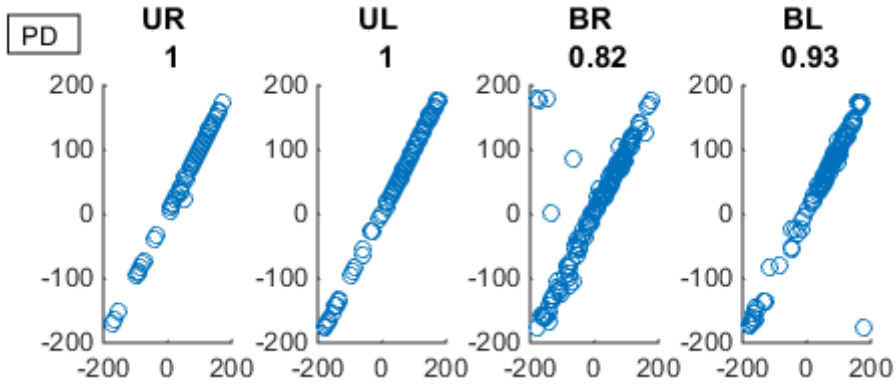


Fig 27: Relationship between the preferred direction values obtained using the cosine-fit and regression without previous averaging. Results are shown for unimanual right, unimanual left, bimanual right and bimanual left trails. Correlation value is shown on the top of each plot.

From the plots we can see a perfectly defined straight line showing a linear relationship between the values obtained for the preferred direction using the two approaches (with and without previous averaging). Furthermore the correlation values are very high and almost exactly one for unimanual movements.

In a second place I conducted a very similar comparison with the tuning amplitude this time. That is, I compared the amplitude coefficients or weights found for each arm for each of the movement types depending on the approach taken for the fit (Fig 28).

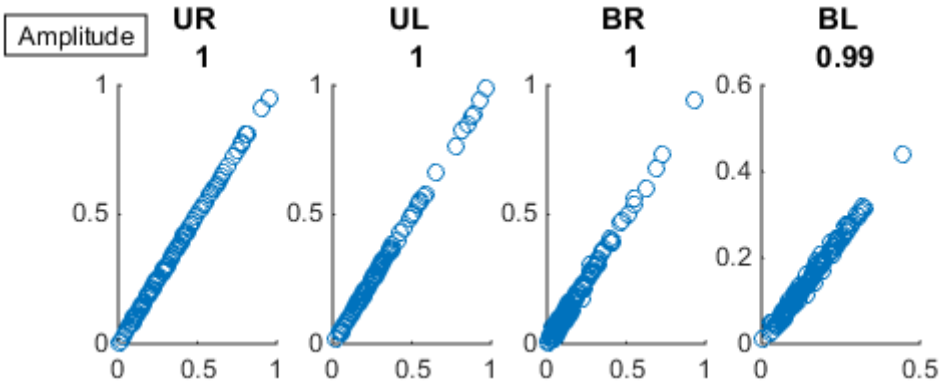


Fig 28: Relationship between the tuning amplitude values obtained using the cosine-fit and regression without previous averaging. Correlation is shown on top of the figure.

Here again we obtain extremely high linear correlations between the tuning amplitude values obtained in each of the methods for both unimanual and bimanual movements.

Finally I wanted to investigate the relationship between the coefficient of determination found for each of the methods. Here again I plotted the values obtained for one approach against those of the other and calculated the correlation between them (Fig 29).

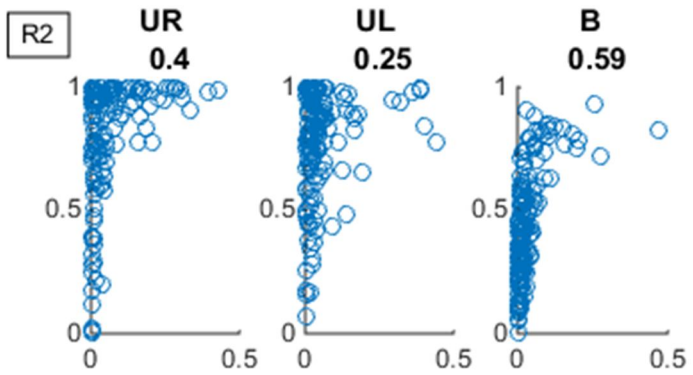


Fig 29: Relationship between the coefficient of determination R^2 obtained using the cosine-fit and regression without previous averaging. R^2 for the approach without previous averaging is shown in the x axis and R^2 for the cosine-fit is shown in the y axis. Correlation is shown on top of the figure.

In this case we get very different results when compared to those obtained for the preferred direction and the amplitude. Here we are not able to visually identify a linear relationship and the correlation between the values obtained for each of the methods is quite low and only larger than 0.5 for bimanual movements. Furthermore we can see that most of the neurons cluster in the upper left corner of the plot: this area corresponds to large R^2 values for the averaging approach and low R^2 values for the non-averaging approach. This means that the vast majority of the neurons have quite a lot lower R^2 values for the non-averaging than for the averaging approach of the fit.

Directional Tuning Assessment

Based on our results and observations we wanted to classify neuronal tuning by means of the tuning depth or regression amplitude instead of the standard coefficient of determination. Similarly as in the standard definition, where a tuned neuron is defined to have a coefficient of determination higher than 0.7, we had to define an amplitude threshold above which the neuron will be considered to be directionally tuned. We wanted to select the amplitude corresponding to a p-value of 0.05 for the multiple linear regression without previous averaging. We therefore studied the relationship between the p-value and the tuning amplitude (Fig 30).

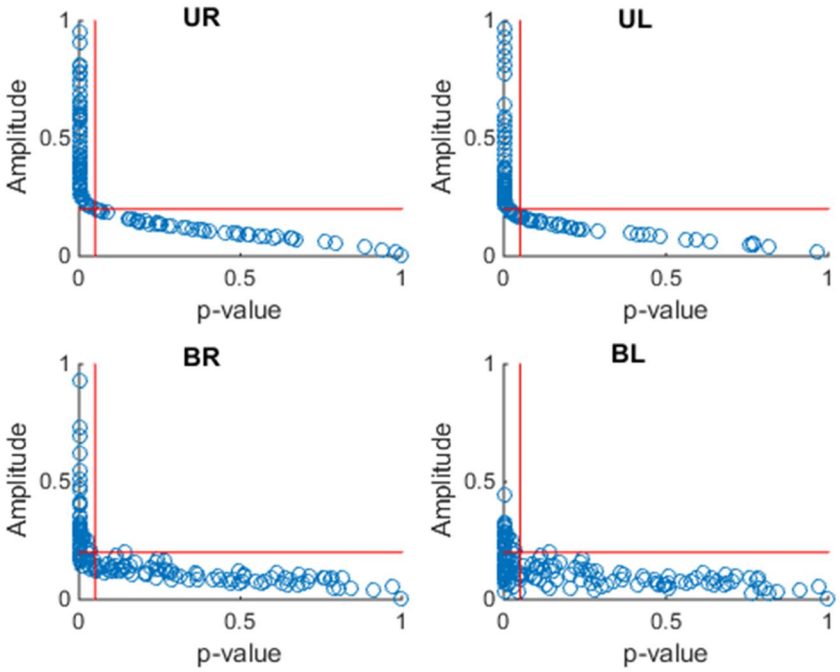


Fig 30: Relationship between the regression amplitude and p-value obtained using the regression without previous averaging. The red lines show the intersection for p-value=0.05 and amplitude=0.2

In the graphs we can see that a p-value of 0.05 approximately corresponds to a tuning amplitude of 0.2. From now on we will thus define tuned neurons as neurons having a regression tuning amplitude greater than 0.2.

I compared the use of this threshold with our regression model to the use of the coefficient of determination with the standard cosine-fit in our neuronal population. In particular I wanted to see whether this new definition for directional tuning is more inclusive or more exclusive than the standard one. For this I compared the percentages of neurons considered to be tuned using each of the two criterions with their corresponding model (Table 3).

% Tuned Neurons	**UR**	**UL**	**BR**	**BL**
R^2 (cosine-fit)	76	69	17	17
Amplitude (regression)	58	61	25	22

Table 3: Percentage of neurons considered to be directionally tuned using each of the two definitions. The percentage is shown for the different movement types (unimaunal right, unimanual left, bimanual right and bimanual left)

The table shows some interesting results. In particular we can see that the percentage of tuned neurons is very similar within movement modes when using the coefficient of

determination: it is around 72% for unimanual movements and 17% for bimanual movements. We can see that this resemblance is maintained when using the amplitude as defining factor: unimanual movements exhibit a tuning percentage close to 60% whereas that of bimanual movements nears 24%. We can also see that the difference in tuning is much higher for unimanual movements in both cases. However we can see that the relative difference between them decreases when using the amplitude as a definition for directional tuning: the percentage of neurons considered tuned in unimanual movements decreases slightly more than 10 points whereas that to bimanual movements increases some 7 points. We can see that despite some slight differences, the major trends and reference values are maintained across the two methods.

Next, we wanted to study how the classification of each individual neuron changes when using a different definition for directional tuning. In particular we have seen that the relationship between the R^2 values is not linear between the two methods. To do this I divided the neuronal population in 20 quantiles corresponding to the directional tuning degree (either measured with the R^2 or the tuning amplitude) and measured the number of quantiles by which each neuron had been displaced when using the two methods. Here is the distribution of the number of quantiles by which each neuron gets displaced when using a different tuning definition (Fig 31).

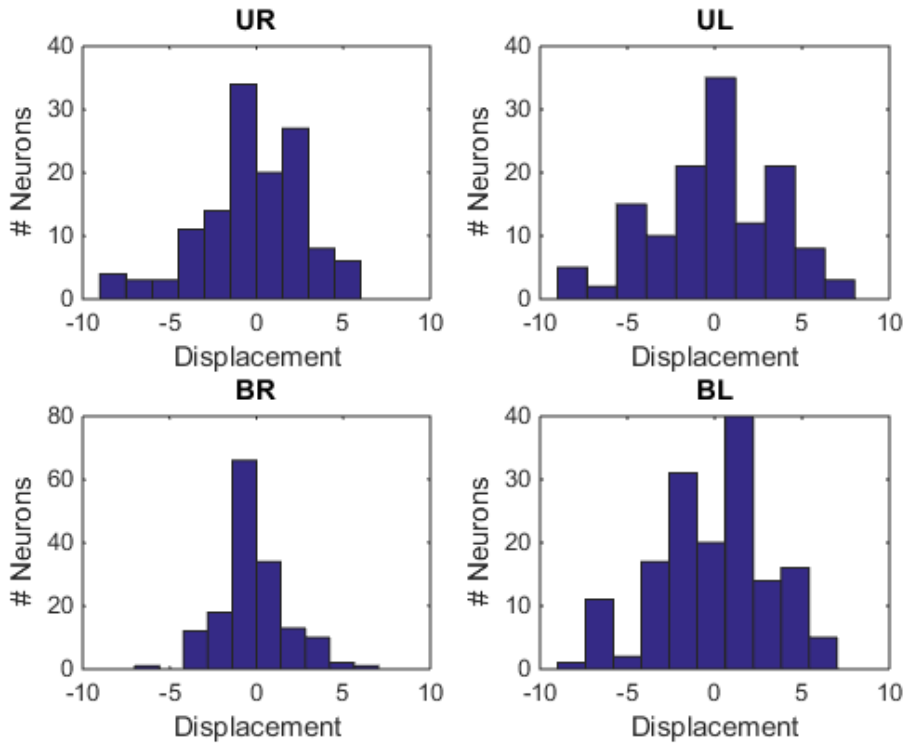


Fig 31: Distribution of the relative position displacement within the neuronal population. Position displacement when using two different methods to determine the directional tuning: tuning amplitude of the regression and cosine-fit R^2. The displacement is given in number of quantiles that each neuron is displaced

We can see that the distribution could look like a noisy Gaussian distribution centered at 0 for all types of movement. This means that the tuning degree assessment of most of the neurons does not vary significantly when using the two different assessment methods. However we see that the amount of outlier neurons is significant in all types of movements except maybe in bimanual right movements. For those neurons the tuning degree assessment is very dependent on the method used to define directional tuning. We plotted a couple of examples where the position of the neuron relative to the neural population when we consider the tuning degree varies a lot depending on the definition used for that assessment (Fig 32).

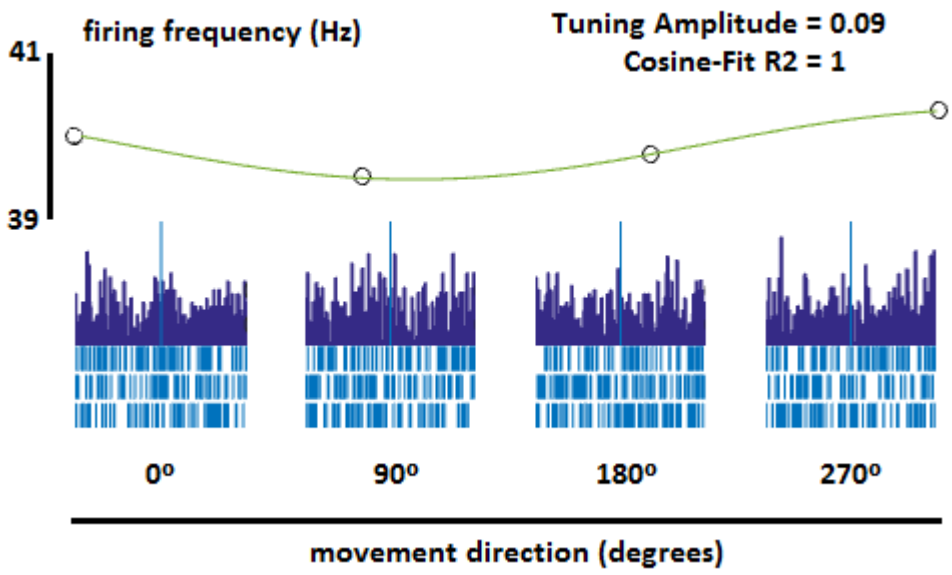


Fig 32: Cosine-Fit, PETHs and raw spiking trains of a very displaced neuron. Neuronal response during unimanual movements in the four directions. The position of this neuron has been lowered by 8 quantiles when using the tuning amplitude definition

In this case we can see that the cosine-fit was extremely accurate and the coefficient of determination was very close to one. However when looking at the PETHs we are not able to identify a clear response to movement or a consistency in a direction preference. We can see that the amplitude obtained for the tuning amplitude when performing the regression is quite low in comparison: 0.09 when the threshold to define directional tuning is 0.2. This is an example of a neuron whose position within the population for the tuning degree has decreased by 8 out of 20 quantiles.

We can look for another example of a cell whose relative position has increased when using the tuning amplitude of the regression as tuning definition.

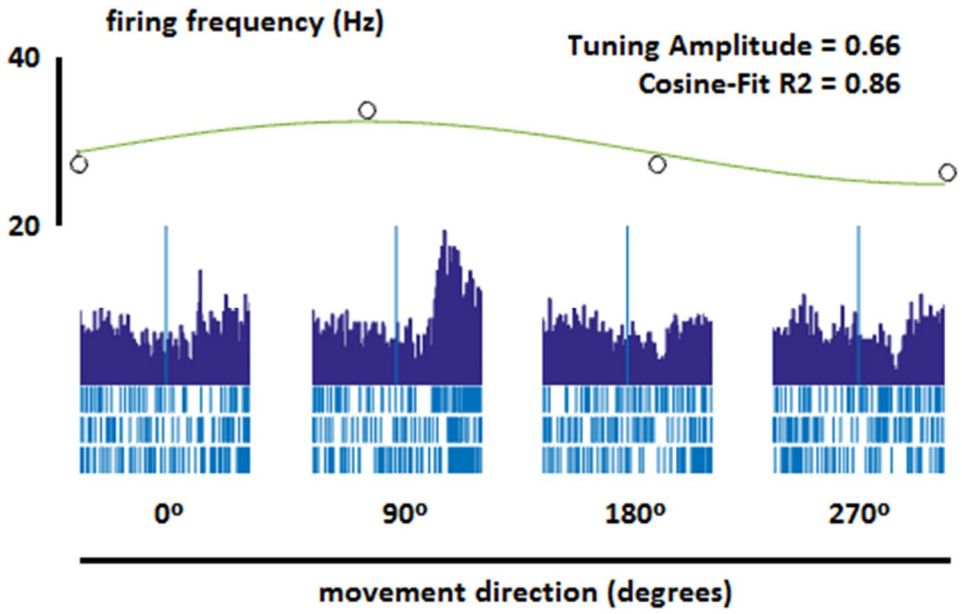


Fig 33: Cosine-Fit, PETHs and raw spiking trains of a very displaced neuron. The position of this neuron has been increased by 6 quantiles when using the tuning amplitude definition

Here we observe that the cosine shape does not perfectly fit the response of the cell. We can see that the coefficient of determination is high although close to the average that is 0.79. When looking at the PETHs however we can see a very clear sensitivity to movement and a sharp increase in the firing rate for movements at 90 degree angles. Therefore the neuron seems to be highly tuned. The tuning amplitude in this case is extremely high and above three times as much as the threshold for tuned neuron definition (which is 0.2). This is an example of a neuron whose relative position within the neuronal population has increased by 6 quantiles.

4.2 Tuning Properties of Unimanual and Bimanual Movements

After analyzing the different procedures that can be undertaken to perform the fits and selecting the most appropriate model, I conducted a study of the properties of directional tuning of the neurons across different movement types. First, I compared the values obtained for the preferred directions in different settings. Second, I tested the linear hypothesis in order

to see whether the neural activation during bimanual movements can be understood as a linear combination of the activation during unimanual movements of the two arms. Finally I analyzed the effects of movement complexity in the tuning degree and the preferred direction of the cells.

4.2.1 Population Tuning Degree for Different Movements

In a first place I studied the degree of directional tuning for the neuronal population as a function of the type of movement. We define the degree of directional tuning within the population as the percentage of neurons that are considered to be directionally tuned. Using the definition of directional tuning described in the previous section, I calculated the percentage of tuned neurons for contralateral and ipsilateral arms as well as for bimanual parallel, opposite and complex movements (Fig 34).

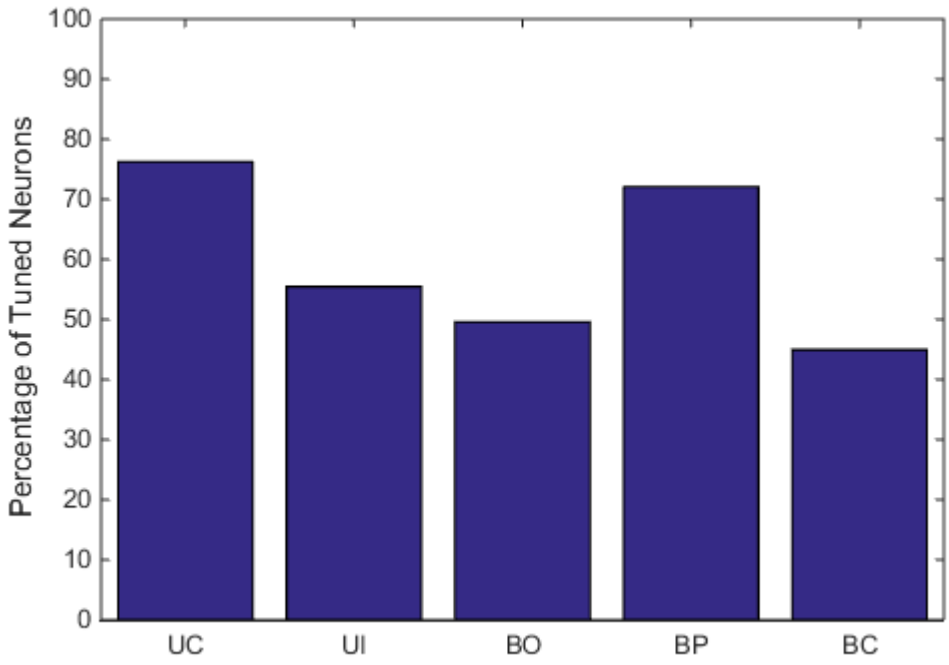


Fig 34: Percentage of directionally tuned neurons during different types of movements: unimanual contralateral (UC), unimanual ipsilateral (UI), bimanual opposite (BO), bimanual parallel (BP) and bimanual complex (BC)

The chart shows some considerable differences for the different movement types. Notably, we can see that contralateral movements show the highest level of tuning within the population

with 76% of the neurons being directionally tuned. Unimanual ipsilateral movements show a much lower percentage of barely 56%. Within the bimanual movements parallel movements have the highest tuning degree. More than 72% of the neurons are tuned during bimanual parallel movements, almost as much as during unimanual contralateral ones. On the other hand bimanual opposite and complex movements show the lowest tuning values: 50% for bimanual opposite and 45% for complex bimanual movements.

4.2.2 Preferred Direction Analysis

After studying the percentage of neurons that was tuned for each movement type I addressed the question of how stable the tuning properties of a neuron are across different movement types. For that I studied first the differences and similarities obtained for the preferred directions and in particular I asked if the preferred direction is maintained for movements of both arms and for both modalities of movement (unimanual and bimanual).

Differences in Right and Left arm Preferred Directions

I first studied the relationship between preferred direction for the left and right arms for both the unimanual and bimanual case. I plotted independently for unimanual and bimanual movements the distribution of differences in preferred directions (expressed in degrees) between the right and left arms for each neuron (Fig 35). Only neurons being defined as directionally tuned for at least one of the arms are included in this plot.

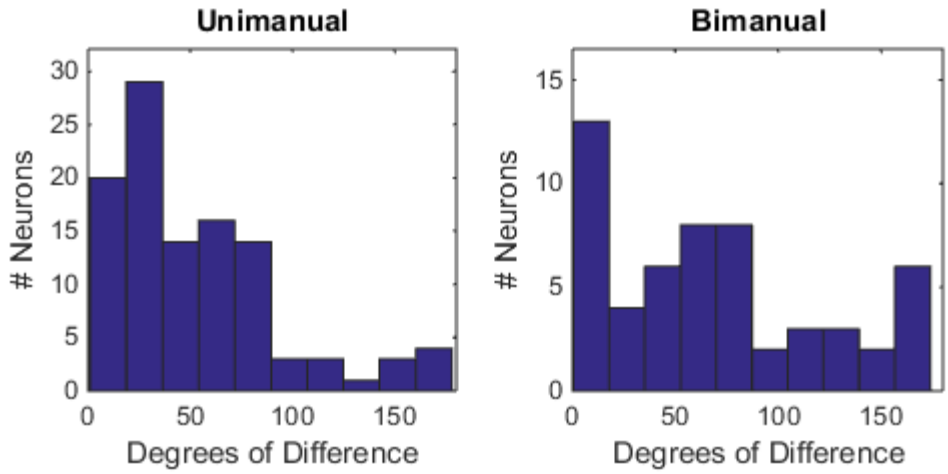


Fig 35: Distribution of the differences in preferred direction between the right and left arms for the directionally tuned neuronal population in unimanual and bimanual movements. The scale of the y axis is chosen as a function of the total number of tuned neurons in each case

We can see a similar distribution in both modalities of movement. In both cases the majority of the neurons exhibit angle differences between the preferred directions of the right and left arms below 90 degrees. This means that neurons tend to have preferred directions for the two arms for both unimanual and bimanual movements that roughly lie in the same region. The differences however are quite important. We should also note that the distribution of differences is wider for the bimanual case, where the values are less clustered around 0. It is interesting to note that the correlation between the differences in preferred direction obtained between the unimanual movements and the bimanual movements is 0.13, a quite low value.

Differences in Preferred Directions between Unimanual and Bimanual Movements

I then investigated whether the preferred direction of a cell for a given arm varied depending on the modality of the movement, that is, depending on it being a unimanual or bimanual movement. For this I plotted the distribution of differences for ipsilateral and contralateral movements (instead of right and left arm movements) and studied the differences (Fig 36).

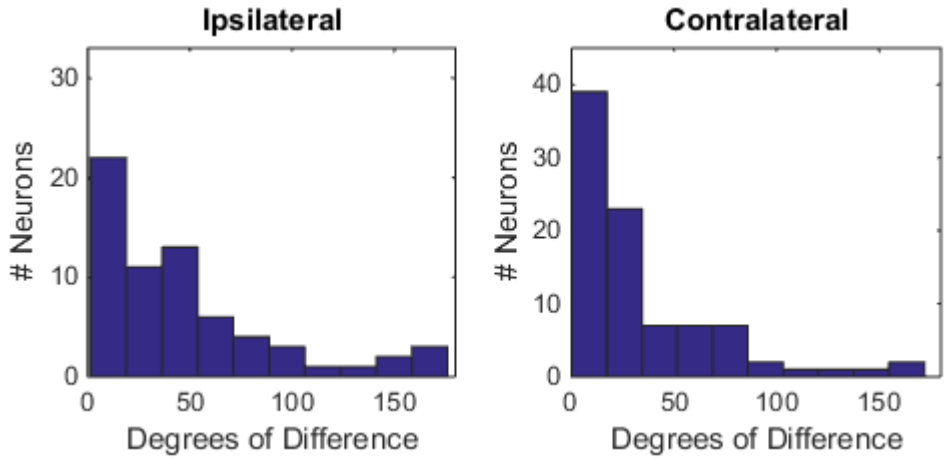


Fig 36: Distribution of the differences in preferred direction between unimanual and bimanual movements for ipsilateral and contralateral arms within the directionally tuned neuronal population. The scale of the y axis is chosen as a function of the total number of neurons considered in each case

In this case we obtain a quite clear result: the majority of the neurons vary just slightly their preferred directions when comparing unimanual and bimanual movements. Contralateral movements show a clear concentration of difference values around 0 degrees. This means that for most of the neurons, the preferred direction of a given neuron during unimanual contralateral movements does not differ much to that obtained when performing bimanual movements. The situation is similar for ipsilateral movements although the concentration of values around 0 degrees is not so strong. This means that the preferred direction of a neuron

when doing unimanual contralateral movements will tend to have just slight variations with respect to bimanual movements, whereas that of the ipsilateral may have larger variations.

Preferred Direction Distributions

Finally I studied the distribution of preferred directions across space. I asked the question whether a neuronal population has a homogenous distribution of preferred directions. For this I first plotted the preferred direction of a given arm as a function of that of the other for both the bimanual and unimanual settings (Fig 37).

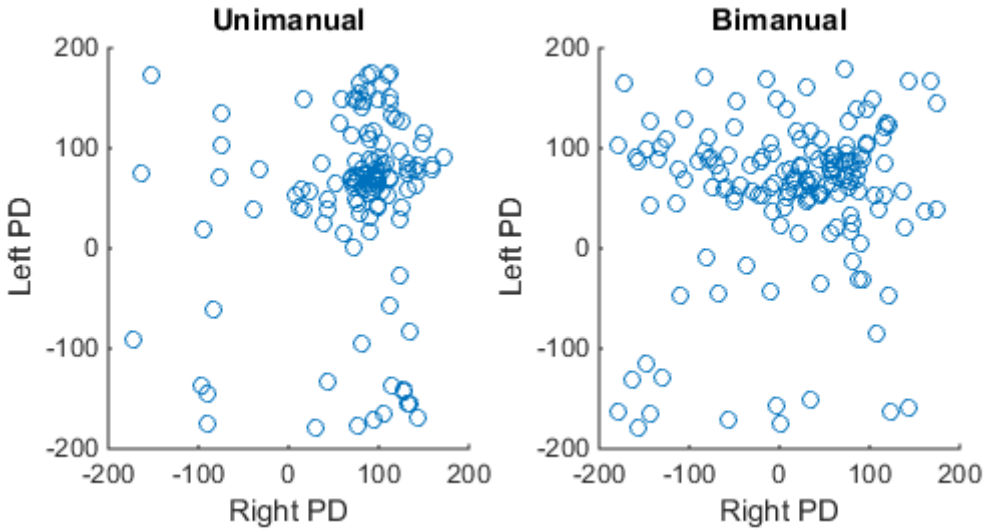


Fig 37: Distribution of Preferred Directions across space. Preferred directions of the right and left for the unimanual and bimanual settings

We can see here a somewhat similar phenomenon for the unimanual and bimanual cases. In particular, most of the neural responses are clustered in the same region. Most of the neurons seem to have values of preferred directions in the range between 50 and 100 degrees for the two arms. The clustering is more noticeable for unimanual movements than for bimanual movements. The range of preferred direction values during bimanual movements is larger for both the right and left arm, although the scattering of values of the right arm seems to be more important than for the left arm.

4.2.3 Linear Hypothesis Testing

In this section I tested the linear hypothesis for neuronal representation of bimanual movements. This hypothesis states that the neuronal activity generated during bimanual movements can be obtained with a linear combination of the activity generated during unimanual movements of each arm.

To assess this I first compared the tuning amplitudes obtained for each neuron in order to see whether the amplitudes obtained for bimanual movements are correlated to those obtained for unimanual movements. I plotted the amplitude obtained for a given neuron in unimanual movements against that of bimanual movements separately for contralateral and ipsilateral movements (Fig 38). All neurons, no matter of their tuning classification, have been included in the plot.

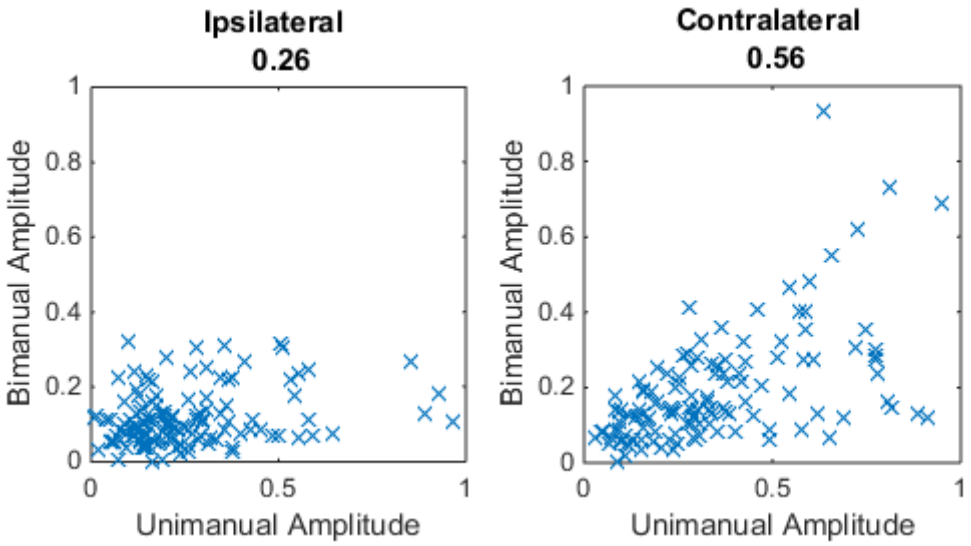


Fig 38: Relationship between the tuning amplitudes of neurons during unimanual and bimanual movements for the ispilateral or contralateral arms. Bimanual amplitudes are shown in the y axis and unimanual amplitudes in the x axis. Correlation between the amplitudes is indicated

We can see that the results are quite different for ipsilateral and contralateral arms. The amplitudes of tuning of neurons during bimanual movements for the ipsilateral arm are scattered and do not show clear correlation with the amplitudes obtained for unimanual movements. The correlation is 0.26, a relatively low value. On the other hand contralateral amplitudes are more clustered and linearly organized. The correlation between contralateral unimanual and bimanual amplitudes is 0.56, a value considerably higher than for the ipsilateral case. To appreciate the differences more clearly I plotted the histograms of amplitude values

for the ipsilateral and contralateral arm separately in unimanual and bimanual movements for all neurons, no matter of their tuning classification (Fig 39).

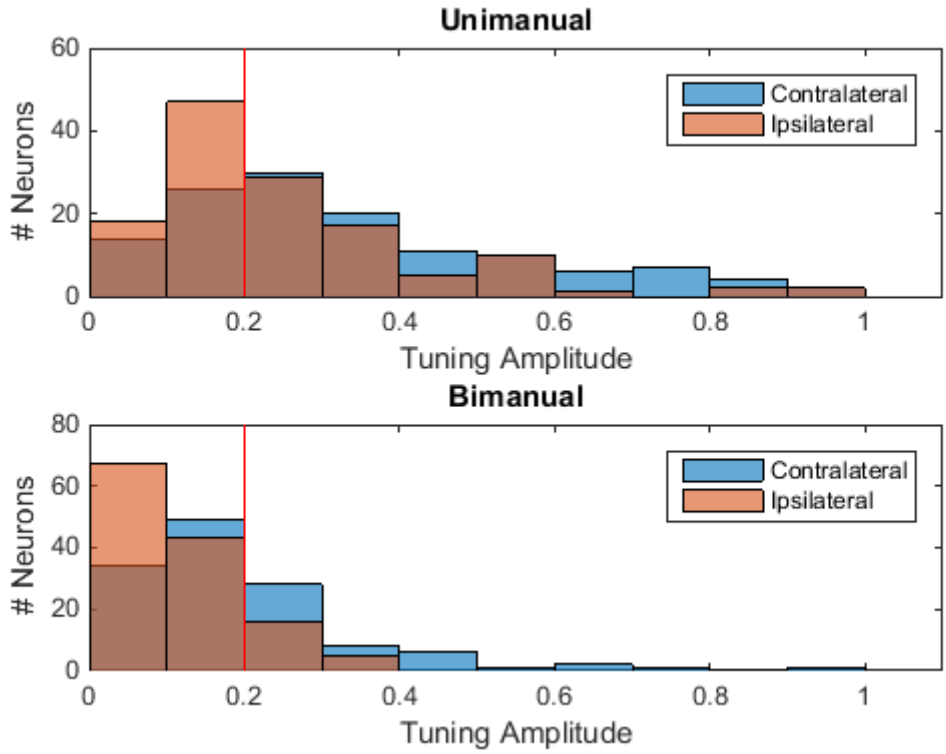


Fig 39: Distribution of tuning amplitude values of the ipsilateral and contralateral arms in unimanual and bimanual movements. The red line shows the threshold value above which a neuron is considered to be directionally tuned

This graph shows us several things. In a first place we can see that the average tuning amplitude for bimanual movements is considerably lower than for unimanual movements. In particular, the majority of the neurons show bimanual amplitudes below 0.3 whereas only a small fraction of the neurons have comparable tuning amplitudes during unimanual movements. In a second place we can observe a difference between amplitudes obtained for contralateral and ipsilateral arms in both unimanual and bimanual movements. Specifically, we can see that in the two cases contralateral amplitude tends to be higher than ipsilateral amplitude.

After studying the relationship between tuning amplitudes I conducted four studies to assess the validity of the linear hypothesis. For this I tested four hypotheses regarding the neuronal responses for bimanual movements: they are equal to the unimanual contralateral response, they are equal to the unimanual ipsilateral response, they are equal to the sum of both unimanual responses or they result from a linear combination of the unimanual responses. I

compared the results obtained assuming each of these hypothesis with the actual bimanual responses and calculated the correlation between them (Fig 40).

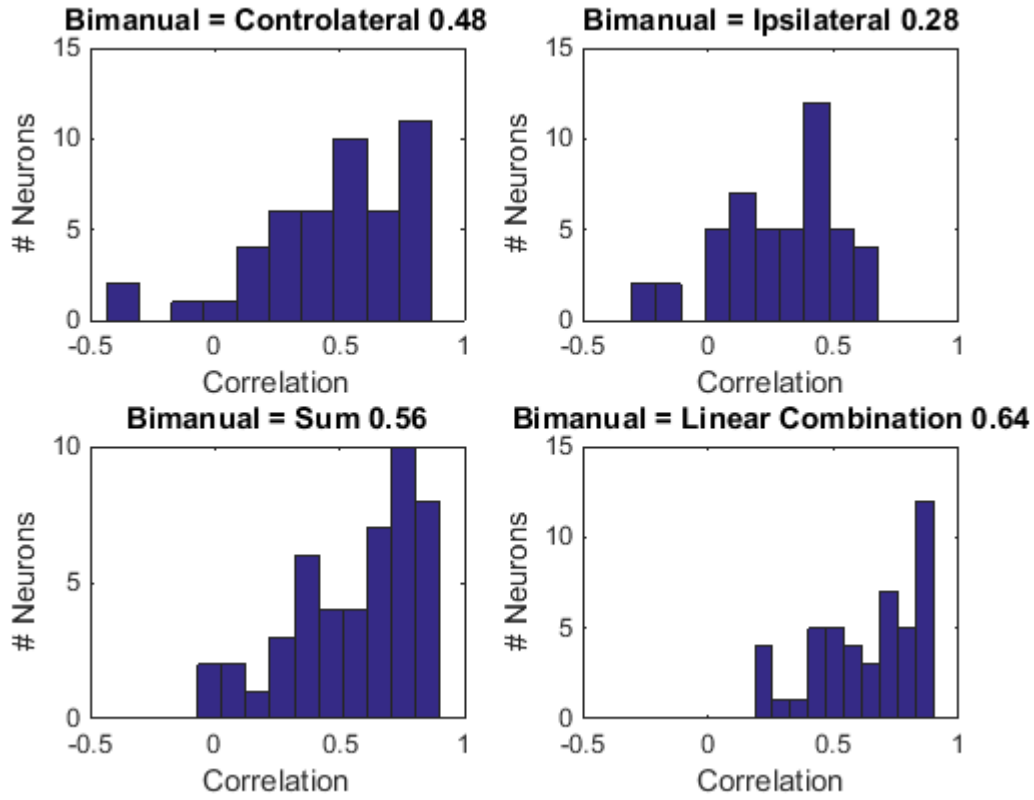


Fig 40: Distribution of correlation values between the results obtained assuming the different hypothesis and the actual bimanual responses. The average correlation is shown above each histogram. Only neurons directionally tuned for unimanual movements with both arms have been included

There are several things to comment here. In a first place we see differences between the correlation values obtained for the ispilateral and contralateral hypotheses. We see that the correlation values are considerably lower for the ipsilateral hypothesis than for the contralateral hypothesis. When looking at the other two hypotheses, sum and linear combination, we can see that the mean correlation values obtained are higher than for any of the contralateral and ipsilateral hypotheses. Specifically the highest correlation is obtained for the case where we assume that bimanual movements correspond to a linear combination of unimanual movements.

4.2.4 Effects of Movement Complexity

I addressed here the question of how the complexity of the movement affects the representation of bimanual movements in the brain. For this I studied in a first place how the preferred direction for each arm and each movement type varies for the case of complex movements (Fig 41). We should note that the same has been done above for the totality of bimanual movements. Because of the characteristics of our regression, where parallel and opposite movements are regressed only against the angles of the contralateral arm, I will only be able to consider the complex case.

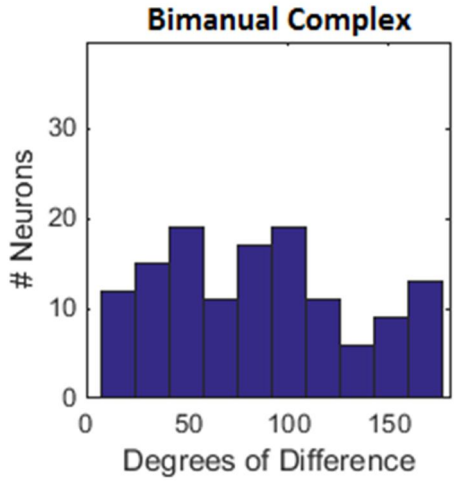


Fig 41: Distribution of the differences in preferred direction between the right and left arms for the directionally tuned neuronal population in unimanual and bimanual complex movements

The distribution shows that the difference in preferred direction between the right and left arm during bimanual complex movements has practically a uniform distribution from 0 to 180 degrees. We should note that this result is quite different from the ones obtained above for the totality of bimanual movements.

Finally I studied the linear hypothesis described above for the different types of bimanual movements: parallel, opposite and complex. I proceeded in the same way as for all bimanual movements together and plotted the distribution of correlations between the actual bimanual movements and the simulated responses if each of the hypotheses is used.

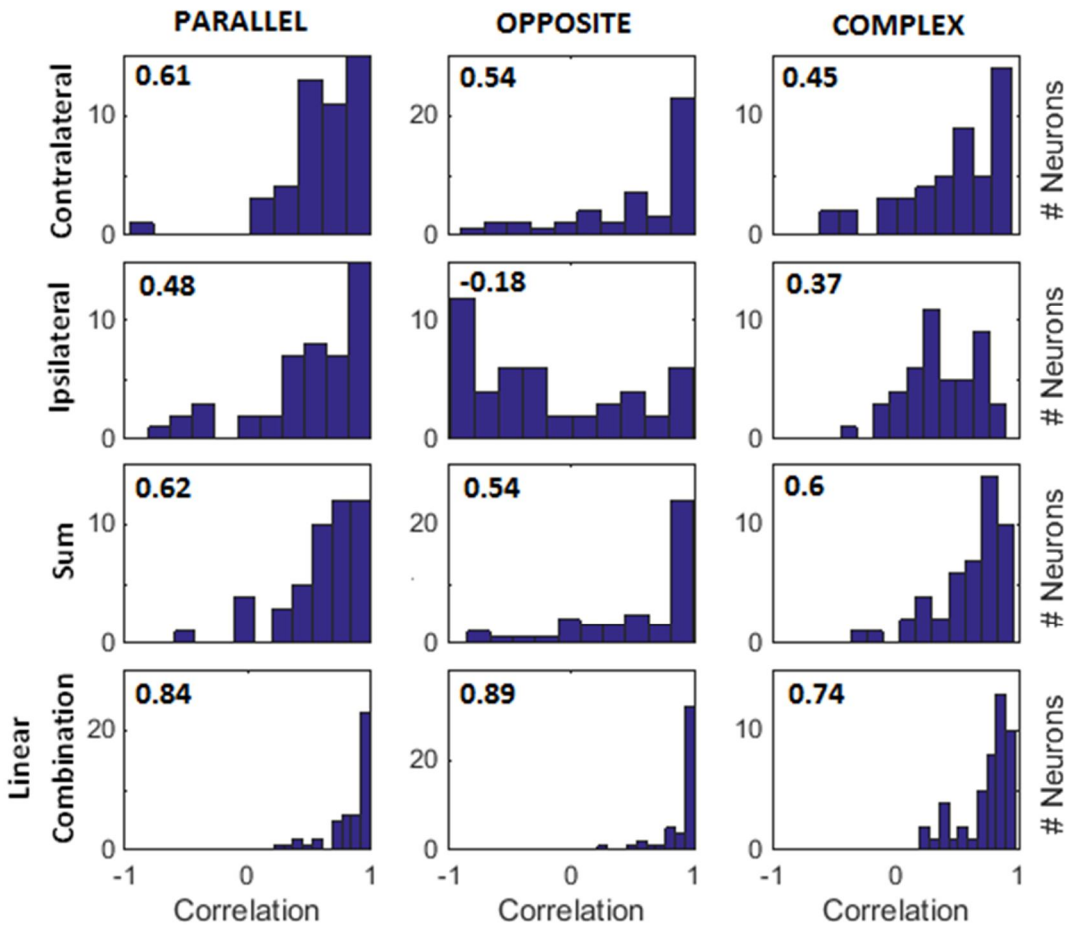


Fig 42: Distribution of correlation values between the results obtained assuming the different hypothesis and the actual bimanual parallel, opposite and complex responses. The average correlation is shown above each histogram. Only neurons directionally tuned for unimanual movements with both arms have been included

There are several things to say from this result. In a first place we have to note that from the two hypotheses stating that the response to bimanual movements is equal to the response of unimanual arms of the contralateral or ipsilateral arms, the contralateral hypothesis gives systematically higher correlation values than the ipsilateral one. When considering the summation hypothesis, we can see that this one slightly outperforms the contralateral hypothesis for parallel movements, considerably outperforms it for complex movements and is equivalent to it for opposite movements. Finally we can see that the linear combination hypothesis works better than any of the other three hypotheses in all bimanual movements. Another important aspect of the graph is the respective variations of the accuracy of the ipsilateral and contralateral hypothesis as a function of the type of bimanual movement. In particular we can see that the highest difference is obtained for opposite movements where the average correlation is 0.54 for the contralateral hypothesis and that of the ipsilateral hypothesis is negative and as low as -0.18. This difference is smaller for the other types of

movement: only 0.13 points separate the accuracy of the two hypotheses in parallel movements and the difference is minimal for complex movements where the contralateral hypothesis is only 0.08 points higher than the ipsilateral one.

Summary

In the first part of this section we studied different methods to analyze neuronal responses: cosine-fit and regression without previous averaging. We found that the tuning values obtained for the preferred direction and amplitude were extremely similar for both the cosine-fit approach and the regression without previous averaging. However we observed that R^2 values varied a lot from one method to the other. Thereafter we developed a new definition for directional tuning based on the regression without previous averaging. This definition is based on the p-value and amplitude obtained for the regression. Finally we studied how this change in definition affected the apparent tuning characteristics of the neurons, and we carefully looked at neurons whose relative position within the neuronal population when considering the tuning degree varied a lot. We were able to see that for those cases, the cosine-fit approach seems to give misleading results. For this reason further studies will be performed with the multiple linear regression without across-trial averaging. Furthermore in order to express the responses as a signal-to-noise ratio the responses for a given neuron will be divided by its standard deviation before performing the regression.

In the second part of this section we analyzed the properties of directional tuning of cortical neurons. First, we found that the preferred direction of a given neuron differs significantly for the two arms, but usually from values below 90 degrees. Then we observed that the preferred direction during unimanual movements is highly conserved in bimanual movements for the contralateral arm. Second, we studied four different hypotheses concerning the linearity of the bimanual response. We found that the bimanual responses are significantly correlated with the unimanual contralateral responses, but a linear combination of the two arms is a better predictor of the actual bimanual response. Finally we studied the effects of movement complexity in the neural representation. Here we observed that for the case of complex bimanual movements, the preferred direction between the two arms considerably varies. Furthermore we tested the linear hypothesis for the different bimanual movement types and observed that the linear combination hypothesis holds better for simple parallel and opposite bimanual movements than for bimanual complex movements.

5 Discussion

5.1 Linear Models of Neuronal Directional Tuning Properties

In this study I analyzed activity patterns of monkey cortical neurons. For each neuron, I determined whether it was tuned to movement direction, and if it was, I determined the preferred direction. In my analyses I used several methods: the standard cosine-fit approach that relied on averaging across behavioral trails corresponding to a particular movement direction, and the regression where no such averaging was conducted. Both methods yielded preferred direction for each neuron, as well as its tuning amplitude, baseline firing rate and coefficient of determination for the fitting.

5.1.1 Cosine-fit Approach

I started with fitting directional tuning curves to trial-average data for different directions. This method is popular in the neurophysiological community because it allows to easily display the dependence of neuronal response on movement direction. An important issue to be noted here is that this method, which include a previous averaging of the all responses obtained for a given target location, can lead to R^2 values that mis-represent how well a neuron represents direction. This observation will be further discussed in the next section.

5.1.2 Regression Model without Preceding Averaging

I then analyzed the results obtained with a regression method where no averaging across trials was done before the regression. The parameters obtained for the tuning properties of the neurons were extremely similar to those obtained with the standard cosine-fit. However the coefficient of determination of the fit had larger values than that of the regression without previous averaging. This means that the standard cosine-fit extracts the same tuning information than the regression without averaging but may lead to an incorrect characterization of neuronal tuning depth. This result is expected since in the second approach we are including all neuronal noise in the regression. This noise causes lower R^2 values.

The study of how neurons would be ranked within the neuronal population as a function of their directional tuning depth when a regression without averaging is used, gives some interesting information. Most of the neurons get a tuning rank that is similar in both methods. However there are some outliers: cases of neurons whose relative position within the population either increases or decreases a lot. This result is expected since for many neurons the coefficient of determination of the standard cosine-fit accurately represents its tuning characteristics. However we can distinguish two cases for which this approach does not work: a neuron non-tuned to movement whose averaged noisy response perfectly fits a cosine by chance, and a neuron that shows a sharp increase in the firing frequency for one of the directions but does not exhibit a well-defined cosine shape. In both of these cases the R^2 will not be representative of the directional tuning properties of the neurons. Examples of this can be seen in the results section.

Taking into account that noise is an essential feature of neurons, it should be taken into account to better understand the role played by neurons in encoding different actions. In particular, high levels of noise during a certain action indicate a low specificity of that neuron to that particular action. Therefore considering noise in the model and comparing it with the signal is very important to correctly assess the responsiveness of neurons to movement direction. On the other hand the cosine-fit approach is commonly used because it is traditionally found in literature. The idea that neuronal response to movement direction corresponds to a cosine was introduced by Georgopoulos [3]. This method is very useful for display purposes because it allows appreciating the overall neuronal response. However the selection of the cosine shape is arbitrary and it is possible that many neurons don't accurately

exhibit such shape in their responses. We have seen the case where the increase in firing rate happens for a tiny range of directions and therefore they do not fit well to a cosine.

Based on this, we believe that the regression model without previous averaging has two major advantages over the standard cosine-fit approach:

- Neuronal noise is preserved
- Regression analysis is directly applied to the trial data instead of average tuning curves

For these reasons we think that this approach could be more accurate for the assessment of which neurons are related to movement and which ones are not. This distinction is important both for theoretical and practical purposes. Firstly it is important in research studies to properly define which neurons are responsive to movement in order to derive accurate properties of neuronal behavior during movement. The inclusion of neurons that do not actually respond to movement might lead to inaccurate conclusions. Secondly, a proper neuronal classification could help improving BMIs by allowing researchers weight the contribution of neurons for decoding as a function of their tuning properties: the higher the directional tuning, the more a neuron should contribute to the decoding algorithm.

In order to determine a new definition of directional tuning based on our regression model I plotted the tuning amplitudes against the p-value obtained for each neuron. We defined a new threshold for the definition of directional tuning based on the p-value. This new definition allows selecting neurons for which the regression is statistically meaningful. We observed that neurons with a p-value below 0.05 have tuning amplitudes above 0.2. For this reason our new definition for directionally tuned neuron can be understood as follows: a directionally tuned neuron is defined as a neuron whose firing rate variability is at most five times the amount of firing rate variation explained by changes in movement direction.

We compared the percentages of tuned neurons that this new definition gives to those obtained with the standard coefficient of determination of the cosine-fit. As we saw, the percentages are similar and therefore we can say that the tuning amplitude threshold is an appropriate value selection to compare results to previous studies using the cosine-fit method.

A supplementary study was performed between the cosine-fit approach and a regression with across-trial averaging. For the latter approach, the same averaging was done preceding the fit, but instead of using the fit, a regression using the target coordinates as predictors was conducted. We obtained that the directional tuning parameters obtained for the two methods, including the coefficient of determination, are almost identical. Some results can be seen in

the Annex (Annex 5). This shows that the major differences existing between the cosine-fit model and the regression without preceding averaging are due to the inclusion of neuronal variability, and not to the fit performed.

5.2 Neuronal Tuning for Unimanual and Bimanual Movements

The major goal of the project was to get some insight into how bimanual movements are represented in the brain and how this representation relates to the response of neurons to unimanual movements

5.2.1 Population Tuning Degree for Different Movements

When calculating the percentage of tuned neurons during different movement types I found that unimanual contralateral movements have a higher tuning degree than unimanual ipsilateral movements. This means that neurons describe a clearer and more specific response to movement direction during movements of the contralateral arm. This agrees with the classical view that each hemisphere controls movements of the opposite body side. When considering bimanual movements, I found that the percentage of tuned neurons is higher for parallel movements than for opposite movements. During parallel movements both arms move in the same direction and thus if the preferred direction of both arms is similar (as our results indicate) the neuron will probably have this same preferred direction when performing bimanual parallel movements and the response to direction will be almost as clear as for unimanual movements. On the other hand, opposite movements combine movements in opposite directions for the two arms. Since the two arms seem to have similar preferred directions, the response of the neuron to opposite movements will be composed of an increase in firing rate for the arm whose movement direction is closer to the preferred direction, and a corresponding decrease in firing rate for the other arm. This will result in a complex response that will not easily be identified as directionally tuned.

When comparing our results to a similar study performed by Steinberg et al. in 2002 we can see that the relative percentages are consistent for the different movement types although our results have systematically higher values [17]. This may be due to the difference in directional tuning definition used in the two studies.

Movement type	Cells (n)	Cells (%)
Unimanual, ipsilateral	54	34
Unimanual, contralateral	86	55
Bimanual, parallel	94	60
Bimanual, opposite	76	48

Table 4: Number and percentage of tuned neurons for different movement types [17]

5.2.2 Preferred Direction Analysis

In this section we found that in the unimanual case, the majority of the neurons have similar preferred directions for the contralateral and ipsilateral arm. Indeed, the difference in preferred directions for the two arms was typically below 90 degrees. This difference increases for the case of bimanual movements.

We then observed that for both ipsilateral and contralateral unimanual movements, the preferred direction slightly changed after a transition from unimanual to bimanual movements. Notable, the preferred direction for the ipsilateral arm changed more than the one for the contralateral arm. This means that the representation of the contralateral arm is more stable across motor behaviors (unimanual vs bimanual) than that of the ipsilateral arm.

5.2.3 Linear Hypothesis Testing

One could intuitively think that the response to bimanual movements results in a combination of the individual responses of unimanual movements of each of the two arms. However, we were able to see that the correlation between the bimanual and unimanual contralateral tuning amplitudes is considerably higher than for the ipsilateral arm. This agrees with previous results where we observed that the representation properties of the contralateral arm are more conserved than that of the ipsilateral arm. Furthermore the tuning amplitude of the contralateral arm during bimanual movements tends to be slightly larger than the ipsilateral one which means that the contribution of the contralateral representation is more important.

I tested four different linear hypotheses and studied the correlation between the real bimanual responses and the simulated responses assuming each of the hypotheses to be true. I found that assuming the bimanual representation to be equal to the contralateral

representation leads significantly better results than considering it to be equal to the ipsilateral arm. This again agrees with my other results. However the summation hypothesis outperforms the two previous ones, which means that the bimanual response can arise as the summation of the response to unimanual movements for some neurons. This also shows that the ipsilateral arm is represented in bimanual movements even if its total contribution to the response is lower than that of the contralateral arm. Finally, the linear combination hypothesis leads to the best results. This is expected since this hypothesis includes all of the other cases and many more different combinations. The average correlation here is 0.64 so we can say that the linear hypothesis often gives an estimation of the bimanual response that is quite related to the real bimanual response. However many aspects of the response cannot be explained by this combination.

5.2.4 Effects of Movement Complexity

Finally I studied the effect of the complexity of the movement in its neuronal representation. For that I compared three types of bimanual movements: parallel, opposite and complex.

In a first place I performed the same as above regarding the changes in preferred the direction but just considering complex movements. This could not be done for parallel and opposite movements since their regression was done against the values of only one movement angle, those of the contralateral arm. For complex movements I found that the differences in preferred direction between the two arms are homogenously distributed from 0 to 180 degrees. This result is quite different to the ones obtained above for general bimanual fits, where most of the neurons exhibited low differences. Complex movements requite higher levels of coordination since the direction of both arms is not related. This increase in the complexity might require the integration of more information and thus a larger change in the response of neurons to movement direction. This might explain why we find larger values in changes in preferred directions between the two arms.

At last I tested the linearity of the bimanual response to each of these movements by means of the four linear hypotheses we already used previously. Here we found several interesting results. We found again that the contralateral response is more similar to the bimanual response than the response to ipsilateral movements for all three types of bimanual movements. However the level of similarity between the ipsilateral response and the bimanual one greatly depends on the type of movement. In particular we found that it is quite reliable

for parallel movements and considerably related in complex movements. However when looking at opposite movements we see that the correlation has a negative value. This result can be understood as follows. Our previous results show that the representation of bimanual movements is strongly related to the contralateral arm and less related to the ipsilateral arm. Besides since the ipsilateral arm is moving in the opposite direction than the contralateral arm in opposite bimanual movements, then the ipsilateral arm should predict the exact opposite response of the real one and the correlation would be negative. This reflects the fact that bimanual responses mostly represent contralateral movements. As a final note we can say that the results obtained with the linear combination are highly correlated to the actual bimanual results. However it is important to note the difference in correlation levels between parallel or opposite movements and complex movements. A lower correlation value in the case of complex movements means that such movements cannot be as accurately described with the linear hypothesis as parallel and opposite movements. Therefore the importance of non-linearities is more important in complex movements. As we have already discussed, complex movements require more coordination and therefore possibly need more inputs from higher cortical areas. This will likely result in the observed non-linearities.

Summary

In the first part of the section, we showed that the new approach yields practically the same tuning curve and tuning parameters as the standard cosine-fit. The advantage of using the regression without previous averaging lies in the fact that it evaluates neuronal tuning depth correctly. This is due to the fact that inter-trial variability is taken into consideration and contrasted with variations actually related to movement directionality. This study is important because since the cosine-fit method was presented by Georgopoulos, it has been the golden standard in this kind of neurophysiological study. This method can yield to over-fitting of noise, thus creating false impressions in the tuning response of neurons [26]. Regressing the signal-to-noise ratio of the individual responses instead of the absolute firing rate of averaged responses prevents such problems and better represents neuronal properties. It is important to note however that the cosine-fit approach is very valuable for display purposes.

In the second part of the section we observed that neurons represent the contralateral arm with similar directional patterns during both unimanual and bimanual movements, but the representation of the ipsilateral arm changes considerably after the transition to bimanual movements. This result indicates that the representation of the contralateral arm can be

thought as the main function of a neuron, whereas the representation of the ipsilateral arm is a supplementary function, which reflects the motor context. This description agrees with a hypothesis called the Mechanism of Callosal Inhibition that was proposed a few years ago by Rokni et al. [8]. Rokni argued that there is a selective inhibition of the representation of the ipsilateral arm during bimanual movements, and this inhibition is mediated by callosal projections. Our results extend this idea because they indicate that the ipsilateral arm representation is affected differently for unimanual and bimanual movements. Furthermore we have seen that the representation of complex bimanual movements is very different from the representation of synergetic movements of the two arms. Based on this finding we suggest that neuronal mechanisms of complex and independent movements of two arms are quite different from the mechanisms that underlie synergetic arm movements. In the former case, the brain needs to control two independent movements, whereas in the latter the same motor commands can be utilized by each arm. It would be of interest to analyze in the future how other brain areas contribute to the mechanisms of complex bimanual movements, for example PPC, which is known to contribute to advanced movements planning.

6 Conclusion

The current project is an investigation of directional tuning properties of cortical neurons, recorded mostly in the motor cortex of rhesus monkeys while they performed bimanual or unimanual reaching movements. Previous research has focused mostly on unimanual movements, whereas little work has addressed bimanual movements. Here we investigated cortical representation of bimanual movements.

In the beginning of the project, different tuning models for the tuning properties of cortical neurons were used and their results compared. I started with the analysis of neuronal directional tuning using cosine-fitting of trial-averaged firing rates for different reach angles. This method assumes the cell's response to the movement direction has a cosine-shape with maximum firing value when movement is performed in the preferred direction of the neuron. Furthermore all trials performed at a particular direction are averaged together, which cancels out neuronal noise. With this method we were able to obtain the different tuning parameters: tuning amplitude, baseline, preferred direction and coefficient of determination. Based on the analysis of some of the results obtained and previous knowledge on the stochastic nature of neuron behavior, we decided to try another method where no averaging was performed previous to the fit. With this model I was able to obtain the tuning parameters by fitting the responses of all individual trials, which means that neuronal noise is included in the model. We consider that noise is an important characteristic of neuronal response and should be taken into account when assessing the tuning properties of a neuron. In particular, based on this new model I developed a new definition for directional tuning where the parameter used to define a neuron as directionally tuned is a function of the signal-to-noise ratio. This definition yielded slightly different results than the standard approach but the overall tendencies were

conserved for both methods. In particular the assessment differences were related to neurons whose tuning characteristics were not properly represented by the standard R^2 definition.

In the second part of the project I described how bimanual movements are encoded in the activity of cortical neurons. In a first place I was able to verify with our data the widely accepted fact that neurons are more specific to movements performed with the opposite side of the body. In particular I observed that the tuning properties of the contralateral arm are much better conserved during bimanual movements than that of the ipsilateral arm. I also showed that the more complex a bimanual movement is, the larger the shifts in the tuning parameters of the cell will be when compared to unimanual movements. Finally I analyzed the linearity of the bimanual responses by testing several linear hypotheses. Here I showed that simple bimanual movements can be approximated with relatively high accuracy by linear combinations of the responses to the unimanual movements of each of the arms. However when bimanual movements get more complex, the prediction accuracy of this model decays. This shows the appearance of non-linearities in the cortical representation of complex bimanual movements, probably due to the integration of signals from high-level cortical areas responsible for the coordination and planning of the movement.

The project has therefore achieved two major goals. First, the golden standard method used in previous literature, the cosine-fit approach, has been analyzed in depth. We have seen that this method is very useful for display purposes although it does not capture the totality of the neuron behavior. For this reason we believe it is preferable to use an approach that takes into account neuronal variability and, in this context, the regression model without previous averaging is more adequate. Finally I inferred from the data some characteristics of the neural representation of bimanual movements which agree with previous studies. Due to the small number of papers published relating to bimanual movements, it is important to show that previously published results can be replicated.

7 Limitations and Future Work

We have stated here our reasons to believe that our linear model for directional tuning has some advantages and represents reality more faithfully when compared to other previously used methods. However this work does not give a quantification of the accuracy of each of the methods and we cannot state that one method is better than the other. One way to investigate this would be to implement the results in a BMI system. That is, based on the two definitions of what a directionally tuned neuron is, we could select the more representative neurons and have them have a stronger contribution to the decoding algorithm of a BMI. Comparing the performance of the BMI using each of the two definitions, we could empirically state that one method is superior to the other in that it represents the behavior of neurons more accurately. Another way to verify this would be to develop a neuronal network model and study how well each of the two approaches extracts the properties of the modeled neurons. This method would enable a straightforward quantitative assessment of the fitting methods.

There are also some major limitations in the study we made on the tuning properties of directional neurons. Specifically we have considered in this project that neurons are independent to each other, and have calculated their tuning parameters based exclusively on their individual responses to movement. We know that this is not true, and that we are able to produce thoughts, feelings and actions thanks to the cooperation of neurons in networks. In particular, analyzing the responses of further brain areas would help understand the origin of the non-linearities found in the neural responses of complex bimanual movements. An example of a region that could contribute to the neural response during complex bimanual movements is the PPC, which we know is related to movement planning. Such analysis could not be done in the current project due to the small sample of neurons located in the PPC.

8 Social Impact Assessment

I believe the current project will contribute to society in two major ways. In a first place, we have seen that a better understanding of the representation of bimanual movements in the brain is essential to design more reliable neural decoder algorithms for BMIs. Besides, the use of more accurate models extracting directional tuning information from neurons can further improve such systems as well as allow conducting more reliable neurophysiological studies. We have seen that BMIs offer a wide range of possibilities for people for neurological disorders. A clear example of people that could benefit from this technology are people with tetraplegia, which just by thinking, would be able to perform actions that are otherwise impossible for them. However we must note that this technology is still very expensive and this is a major limitation for individuals. In particular, this kind of technology sets a clear barrier between developed and developing countries.

This kind of study is important for our knowledge too. The brain has long been a mystery for us and still we do not really understand how information is processed in the brain. In this context, understanding in detail how cortical cells generate movement commands to the muscles is critical. Furthermore, this knowledge might open the door for understanding more complex processes that are based on the same principles.

9 Budget

Human Labor Costs

Status	Number	Cost (€ per month)	Time Investment (hours)	Cost (€ per project)
External Supervisor	1	2500	75	1040
Internal Supervisor	1	2500	50	690
Biomedical Engineering Student	1	1000	450	2570

Work hours per month: 180

Material Costs

Description	Hours of use	Initial cost (€)	Amortization (€)
Computer	370	1000	100
Software (MATLAB)	280	100	50
Other Material			50

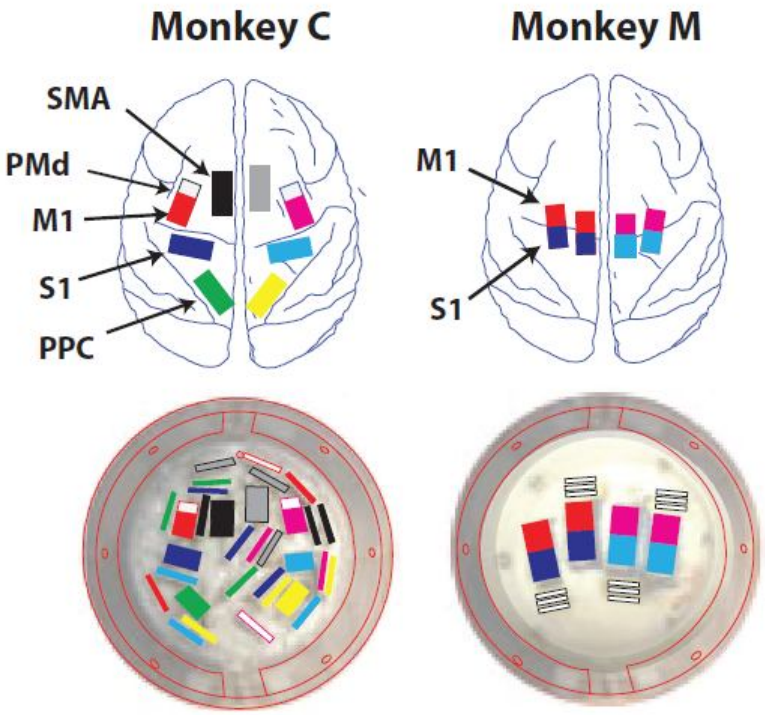
Total costs

Description	Cost (in €)
Material Costs	200
Human Labor Costs	4030
Indirect costs	500
Total (without IVA)	5000
IVA 21%	1049
Estimated Total	**6049**

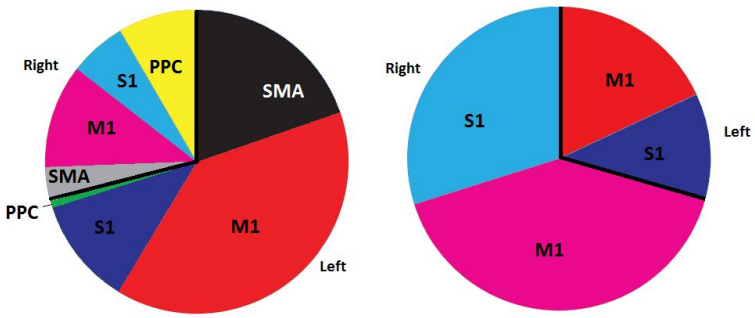
Note: Experiment costs are not included here.

10 Appendices

10.1 Electrode Placement in the Cortex

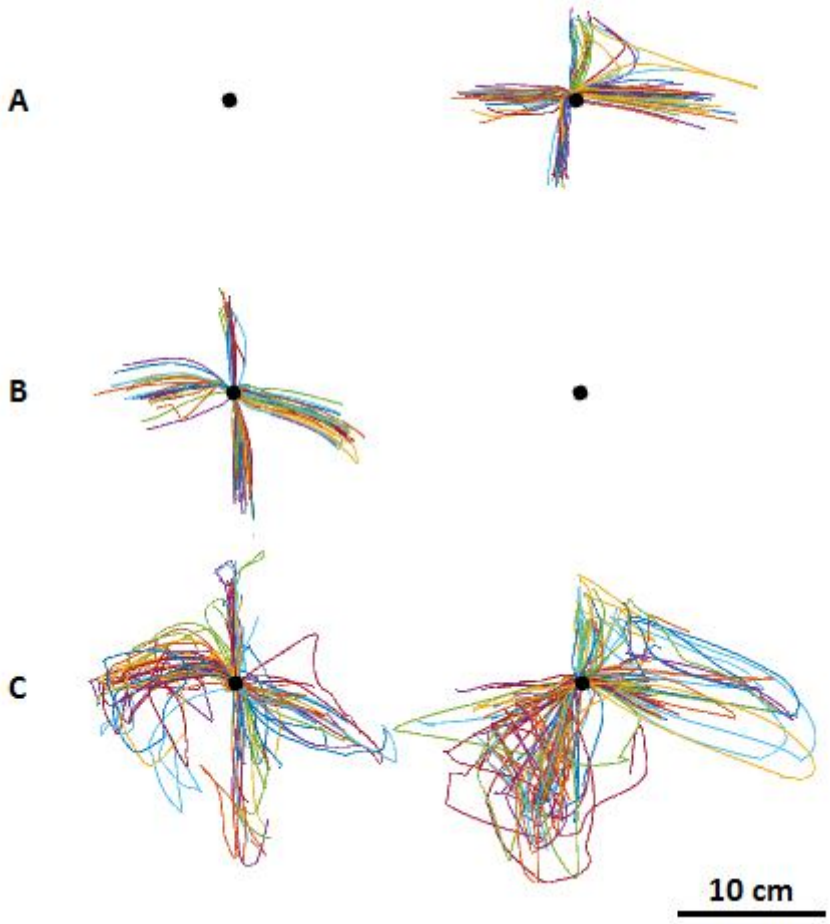


Annex 1: Placement of the electrode arrays in the cortex of the monkeys

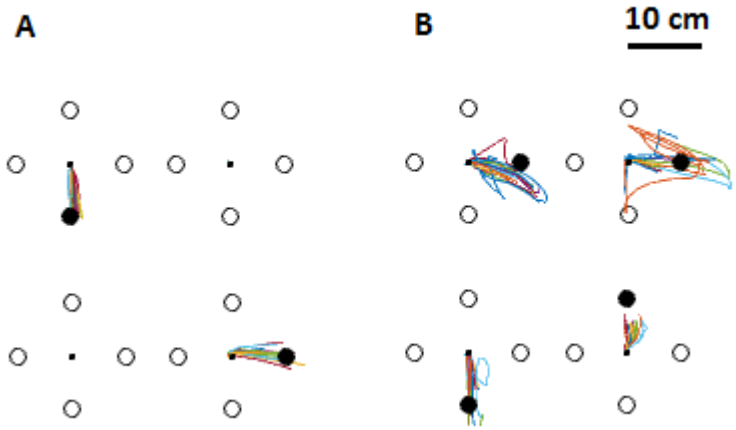


Annex 2: Distribution of electrodes across the cortex

10.2 Movement Trajectories



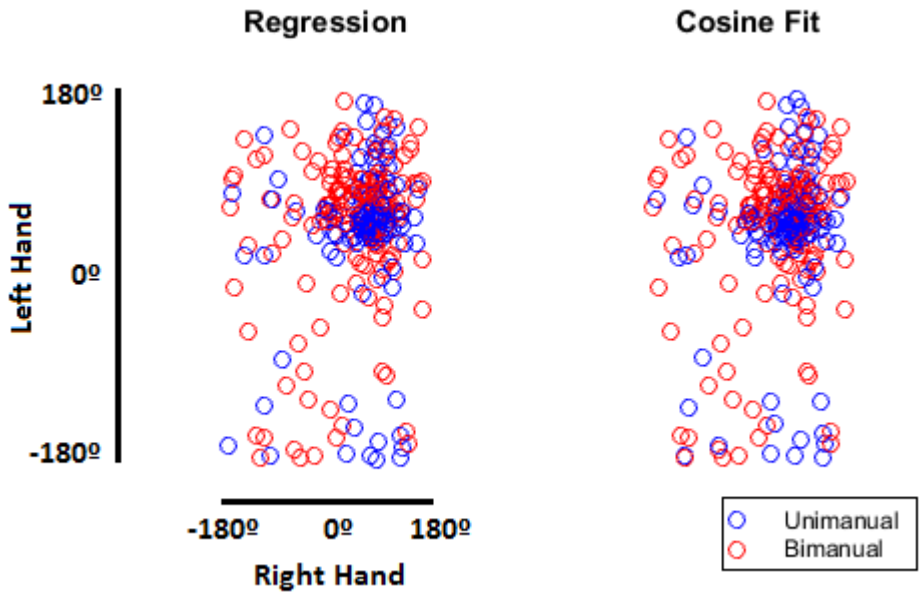
Annex 3: Movement trajectories of the monkey for A. Unimanual Right movements, B. Unimanual Left movements, C. Bimanual movements. Movements are plotted from movement onset to reward



Annex 4: Trajectories for several combinations of A. Unimanual and B. Bimanual parallel and opposite movements. The target for the plotted trials is filled in black.

10.3 Regression model with preceding across-trial averaging

We performed a regression with previous across-trial averaging and compared the directional tuning parameters with those obtained with the cosine-fitting. Below is shown the distribution of preferred directions across space for each of the methods. We can see here that the pattern of preferred directions obtained for each of the methods is highly similar. However some minor differences can be appreciated when one looks carefully into the figure. The correlation between the results is 0.97, a high value showing a strong correlation between the two sets of results. Similar observations can be done for the rest of the parameters (i.e. tuning amplitude, baseline firing rate and coefficient of determination for the fit).



Annex 5: Comparison of the distribution of preferred directions for regression and cosine fitting methods with previous averaging of the responses for each target location

10.4 MATLAB Code of the Models

10.4.1 Cosine-Fit Model

Unimanual Movements

```
y=firingRates;
angles=[2*pi 3*pi/2 pi pi/2]';
y=zeros(4,1);
for i=1:4; y(i,1)= mean (y(targetIndex==i));end
f = fittype('a*cos(x-b)+c');
[mycurve,gof]=fit(angles,y,f,'StartPoint',[1 1 1]);
r2=gof.rsquare;
prefDirection=mycurve.b;
amplitude=mycurve.a;
baseline=mycurve.c;
if mycurve.a<0;
prefDirection=prefDirection-pi;amplitude=-(amplitude);
end
prefDirection = ppalAngle(prefDirection);
```

Bimanual Movements

```
y= firingRates;
angle1=[0 0 0 0 -pi/2 -pi/2 -pi/2 -pi/2 -pi -pi -pi -pi -3*pi/2 -
3*pi/2 -3*pi/2 -3*pi/2]';
angle2=[ 0 -pi/2 -pi -3*pi/2 0 -pi/2 -pi -3*pi/2 0 -pi/2 -pi -3*pi/2 0
-pi/2 -pi -3*pi/2]';
y=zeros(16,1);
for i=1:16;y(i,1)= mean (Y(targetIndex==i,1));end
f = fittype( @(c,a1,b1,a2,b2,angle1,angle2) a1*cos(angle1-
b1)+a2*cos(angle2-b2)+c ,'independent', {'angle1', 'angle2'}, ...
        'dependent', 'y' );
[mycurve,gof]=fit([angle1,angle2],z,f,'StartPoint',ones(1,5));
r2=gof.rsquare;
if mycurve.a1<0;
prefDirectionLeft=mycurve.b1-pi;amplitude(1)=-mycurve.a1;
else prefDirectionLeft=mycurve.b1;amplitude(1)=mycurve.a1;
end
if mycurve.a2<0;
prefDirectionRight=mycurve.b2-pi;amplitude(2)=-mycurve.a2;
else prefDirectionRight=mycurve.b2;amplitude(2)=mycurve.a2;
end
prefDirectionLeft = ppalAngle(prefDirectionLeft);
prefDirectionRight = ppalAngle(prefDirectionRight);
baseline=mycurve.c;
```

Bimanual Parallel Movements

```
y=firingRates; Ystd = std(firingRates);
angle=[0 -pi/2 -pi -3*pi/2 ]';
parallel_trials=[1 6 11 16];
y=zeros(4,1);
for i=1:4; y(i,1)= mean (y(targetIndex==parallel_trials(i)));end
```

```
y=y/Ystd;
f = fittype('a*cos(x-b)+c');
[mycurve,gof]=fit(angles,y,f,'StartPoint',[1 1 1]);
r2=gof.rsquare;
prefDirection=mycurve.b;
amplitude=mycurve.a;
baseline=mycurve.c;
if mycurve.a<0;
prefDirection=prefDirection-pi;amplitude=-(amplitude);
end
prefDirection = ppalAngle(prefDirection);
```

Bimanual Opposite Movements

```
y=firingRates;
if strcmp(hemisphere,'R');angle=[2*pi 3*pi/2 pi pi/2]';
elseif strcmp(hemisphere,'L'); angle=[pi pi/2 2*pi 3*pi/2]';
end
opposite_trials=[3 8 9 14];
y=zeros(4,1);
for i=1:4; y(i,1)= mean (y(targetIndex==opposite_trials(i)));end
f = fittype('a*cos(x-b)+c');
[mycurve,gof]=fit(angles,y,f,'StartPoint',[1 1 1]);
r2=gof.rsquare;
prefDirection=mycurve.b;
amplitude=mycurve.a;
baseline=mycurve.c;
if mycurve.a<0;
prefDirection=prefDirection-pi;amplitude=-(amplitude);
end
prefDirection = ppalAngle(prefDirection);
```

Bimanual Complex Movements

```
y= firingRates;
angle1=[2*pi 2*pi 3*pi/2 3*pi/2 pi pi pi/2 pi/2]';
angle2=[3*pi/2 pi/2 2*pi pi 3*pi/2 pi/2 2*pi pi]';
complex_trials=[2 4 5 7 10 12 13 15];
y=zeros(8,1);
for i=1:8;y(i,1)= mean (y(targetIndex==complex_trials(i)));end
f = fittype( @(c,a1,b1,a2,b2,angle1,angle2) a1*cos(angle1-
b1)+a2*cos(angle2-b2)+c ,'independent', {'angle1', 'angle2'}, ...
        'dependent', 'y' );
[mycurve,gof]=fit([angle1,angle2],z,f,'StartPoint',ones(1,5));
r2=gof.rsquare;
prefDirection=mycurve.b;
amplitude=mycurve.a;
baseline=mycurve.c;
if mycurve.a<0;
prefDirection=prefDirection-pi;amplitude=-(amplitude);
end
prefDirection = ppalAngle(prefDirection);
```

10.4.2 Regression Model without Previous Averaging

Unimanual Movements

```
X = targetAngles;
y = firingRates;
y = y/std(y);
X = [cos(X) sin(X) 1];
[b,~,~,~,stats] = regress(y,X);
amplitude = sqrt(b(1)^2+b(2)^2);
baseline=b(3);
r2 = stats(1);
p-value = stats(3);
prefDirection = angle(b(1) + 1i*b(2));
```

Bimanual Movements

```
X = targetAngles;
y = firingRates;
y = y/std(y);
X = [cos(X(:,1)) sin(X(:,1)) cos(X(:,2)) sin(X(:,2))];X(:,5)=ones;
[b,~,~,~,stats] = regress(y,X);
amplitudeLeft = sqrt(b(1)^2+b(2)^2);
amplitudeRight = sqrt(b(3)^2+b(4)^2);
baseline=b(5);
r2 = stats (1);
p-value = stats(3);
prefDirectionLeft = angle(b(1) + 1i*b(2));
prefDirectionRight = angle(b(3) + 1i*b(4));
```

Bimanual Parallel Movements

```
parallel_trials=find(targetIndex==1| targetIndex ==6| targetIndex
==11| targetIndex ==16);
X = targetAngles(parallel_trials,1);
y = firingRates;
y = y/std(y);
y=y(parallel_trials);
X = [cos(X) sin(X) 1];
[b,~,~,~,stats] = regress(y,X);
amplitude = sqrt(b(1)^2+b(2)^2);
baseline=b(3);
r2 = stats(1);
p-value = stats(3);
prefDirection = angle(b(1) + 1i*b(2));
```

Bimanual Opposite Movements

```
opposite_trials=find(targetIndex ==3| targetIndex ==8| targetIndex
==9| targetIndex ==14);
if strcmp(hemisphere,'R'); X = targetAngles(opposite_trials,1);
else X = targetAngles(opposite_trials,2);
end
y = firingRates;
y = y/std(y);
```

```
y=y(opposite_trials);
X = [cos(X) sin(X) 1];
[b,~,~,~,stats] = regress(y,X);
amplitude = sqrt(b(1)^2+b(2)^2);
baseline=b(3);
r2 = stats(1);
p-value = stats(3);
prefDirection = angle(b(1) + 1i*b(2));
```

Bimanual Complex Movements

```
complex_trials=find(targetIndex ==2| targetIndex ==4| targetIndex ==5|
targetIndex ==7| targetIndex ==10| targetIndex ==12| targetIndex ==13|
targetIndex ==15);
X = targetAngles(complex_trials,:);
y = y/std(y);
y = firingRates(complex_trials);
X = [cos(X(:,1)) sin(X(:,1)) cos(X(:,2)) sin(X(:,2))];X(:,5)=ones;
[b,~,~,~,stats] = regress(y,X);
amplitudeLeft = sqrt(b(1)^2+b(2)^2);
amplitudeRight = sqrt(b(3)^2+b(4)^2);
baseline=b(5);
r2 = stats (1);
p-value = stats(3);
prefDirectionLeft = angle(b(1) + 1i*b(2));
prefDirectionRight = angle(b(3) + 1i*b(4));
```

11 Bibliography

[1] Duke Center for Neuroengineering, «Nearly Two Thousand Brain Cells Recorded at One Time,» 2014.

[2] T. Aflalo et al., «Decoding Motor Imagery from the Posterior Parietal Cortex of a Tetraplegic Human,» *Science,* vol. 348, nº 6237, pp. 906-910 , 2015.

[3] A. P. Georgopoulos, «On the relations between the direction of two-dimensional arm movements and cell discharge in primate motor cortex,» *The Journal of Neuroscience,* vol. 2, nº 11, pp. 1527-37, 1982.

[4] Cardoso de Oliveira et al., «Neural interactions between motor cortical hemispheres during bimanual and unimanual arm movements,» *European Journal of Neuroscience,* vol. 14, nº 11, pp. 1881-96, 2001.

[5] J. Rickert et al., «Encoding of Movement Direction in Different Frequency Ranges of Motor Cortical Local Field Potentials,» *The Journal of Neuroscience,* vol. 25, nº 39, pp. 8815-8824, 2005.

[6] A.P. Georgopoulos et al., «Primate Motor Cortex and Free Arm Movements to Visual Targets in Three-Dimensional Space. II. Coding of the Direction of Movement by a Neuronal Population,» *The Journal of Neuroscience,* vol. 8, nº 8, pp. 2928-37, 1988.

[7] R. Tresilian et al., «Common organization for unimanual and bimanual reach-to-grasp tasks,» *Experimental Brain Research,* vol. 115, nº 2, pp. 283-99, 1997.

[8] U. Rokni et al., «Cortical representation of bimanual movements,» *The Journal of Neuroscience,* vol. 23, nº 37, pp. 11577-11586, 2003.

[9] P.J. Ifft et al., «A brain machine interface enables bimanual arm movements in monkeys,» *Science Translational Medicine,* vol. 5, nº 210, 2013.

[10] E. Bianconi, «An estimation of the number of cells in the human body,» *Annals of Human Biology,* vol. 40, nº 6, pp. 463-71, 2013.

[11] K.V.Shenoy et al., «Cortical control of arm movements: a dynamical systems perspective,» *Annual Review of Neuroscience,* vol. 36, pp. 337-359, 2013.

[12] G. W. Schacter, Psychology, New York: Worth Publishers, 2009.

[13] W. Truccolo et al., «Primary Motor Cortex Tuning to Intended Movement Kinematics in Humans with Tetraplegia,» *The Journal of Neuroscience,* vol. 28, nº 5, pp. 1163-78, 2008.

[14] A.P. Georgopoulos et al., «Neuronal Population Coding of Movement Direction,» *Science*

Reports, vol. 233, pp. 1416-19, 1986.

[15] B. Amirikian et al., «Directional tuning profiles of motor cortical cells,» *Neuroscience Research,* vol. 36, pp. 73-79, 2000.

[16] O. Donchin et al., «Single-Unit Activity Related to Bimanual Arm Movements in the Primary and Supplementary Motor Cortices,» *Journal of Neurophysiology,* vol. 88, p. 3498–3517, 2002.

[17] O. Steinberg et al., «Neuronal populations In primary motor cortex encode bimanual arm movements,» *European Journal of Neuroscience ,* vol. 15, nº 8, pp. 1371-80, 2002.

[18] J.M. Carmena et al., «Learning to control a brain machine interface for reaching and grasping in primates,» *PLoS Biology,* vol. 1, nº 2, 2003.

[19] M.A. Lebedev et al., «Cortical ensemble adaptation to represent actuators controlled by a brain machine interface,» *The Journal of Neuroscience,* vol. 25, nº 19, pp. 4681-93, 2005.

[20] L.R. Hochberg et al., «Reach and grasp by people with tetraplegia using a neurally controlled robotic arm,» *Nature,* vol. 485, p. 372–375, 2012.

[21] P. Campbell, «Amputee Makes History with APL's Modular Prosthetic Limb,» *Johns Hopkins University,* 2014.

[22] L. Hochberg, «Neuronal ensemble control of prosthetic devices by a human with tetraplegia,» 2006.

[23] W. Wu et al., «Modeling and decoding motor cortical activity using a switching Kalman filter,» *IEEE Transactions of Biomedical Engineering,* vol. 51, nº 6, pp. 933-42, 2004.

[24] Z. Li et al., «Unscented Kalman Filter for Brain-Machine Interfaces,» *PLoS ONE,* 2009.

[25] M. M. Churchland et al., «Neural population dynamics during reaching,» *Nature,* vol. 487, p. 51–56, 2012.

[26] K. Ganguly et al., «Emergence of a Stable Cortical Map for Neuroprosthetic Control,» *PLoS Biology,* vol. 7, nº 7, 2009.

[27] J. M. Abdullah, Summary of Readings in Neurosurgery BRAIN, 2013.

[28] M. S. Gazzaniga, «Cerebral specialization and interhemispheric communicationDoes the corpus callosum enable the human condition?,» *BRAIN A Journal of Neurology,* vol. 123, pp. 1293-1326, 2000.

[29] M. Nicolelis, Beyond Boundaries, New York: Times Books, 2011.